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OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

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VOLUME VI—1919

WITH FORTY-ONE PLATES AND NINETY-SIX TEXT FIGURES

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT 41 NORTH QUEEN STREET, LANCASTER, PA.

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

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(Dates of publication: No. 1, Feb. 3; No. 2, Mar. 1; No. 3, Apr. 28; No. 4, May 1; No. 5, June 20; No. 6, July 21; No. 7, Aug. 16; No. 8, Nov. 22; No. 9, Dec. 18; No. 10, Jan. 7.)

ERRATA, VOLUME VI

Page 203, line 10, read Kran**z**lein.
Page 206, line 4 from bottom, vol. *1*, read **7**.
Page 212, line 3 under C. macrantha, for pe*t*alis, read pe**d**alis.
Plate XXX, for limosi**folia**, read limosi**flora**.
Page 243, line 1, footnote [1] should read [6].
Page 244, line 4, footnote [1] should read [7].

Vol. VI. JANUARY, 1919 No. 1

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Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania,
under the act of March 3,1 879

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DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

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The Journal is published monthly, except during August and September. Subscription price, $5.00 a year. Single copies 60 cents, plus postage. Postage will be charged to all foreign countries, except Mexico, Cuba, Porto Rico, Panama, Canal Zone, Republic of Panama, Hawaii, Philippine Islands, Guam, Samoan Islands, and Shanghai. Postage to Canada 20 cents a volume on annual subscriptions; to all other countries in the Postal Union, 40 cents a volume on annual subscriptions.

The pages of the Journal are open to members of the Botanical Society of America, or candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $2.50 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Author may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $4.50 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Fifty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence, including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or 41 North Queen Street, Lancaster, Pa.

AMERICAN
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VOL. VI JANUARY, 1919 No. 1

Smithsonian Institution
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BIOLOGIC SPECIALIZATION IN THE GENUS SEPTORIA

WALTER SPURGEON BEACH

INTRODUCTION

The genus Septoria presents an excellent field for the study of biologic specialization on account of the very large number and the uncertain status of the described species, of which there are more than 1200. The morphological characters for the limitation of these are few, consisting chiefly of spore shape, length, thickness, and septation, and in some instances a slight tinting of the spore; pycnidium shape, size, and color; and ostiole size and character. The biological characters for the limitation of species are the size, shape, margin, zonation, and color of the disease spots, and their location and distribution upon the host. The specific descriptions in many cases are meager and really useless. The presence of a Septoria upon an unrecorded host is often made the basis of a new species.

By tabulating (10), according to spore length, all the "species" of Septoria given in Saccardo's "Sylloge Fungorum," it has been found that nearly 700 fall within the limits of 20 to 50 μ. Twenty-six species on grasses are between 20 and 40 μ. Yet in one case a Saccardian description gives a spore length ranging from 19 to 62 μ. There have been great inaccuracies in measuring spores and pycnidia, with tendencies to report the measurements in round numbers. Elliott (7) has shown the degree of personal error with our modern technique. Among eleven observers measuring many spores of Alternaria, he found that there was a variation of over 41 per cent from the highest measurement returned for maximum length, and that such a variation would make it impossible to distinguish the species of Alternaria on the basis of spore measurement. Septation is largely dependent upon the stage of maturity of the spores, is often difficult to see, and was ignored by early mycologists. Environmental conditions cause fluctuations in the size of spores and pycnidia. The value of disease characters is doubtful, for they commonly change with the age, the part, and the species of the host, as well as with weather conditions. The confusion that Cavara (4) met regarding these points in his attempt to distinguish *Septoria tritici* and *S. graminum* on wheat, led him to state that both forms probably belong to the same morphological species.

Little is known respecting the existence of biologic specialization in the genus Septoria. Except for a few instances (15, 22, 45), no cross-inoculations have been made to ascertain whether a Septoria, as found upon a particular host, is restricted to that host, or is more or less cosmopolitan and adaptive in its parasitism. In the literature at present one is confronted by two extremes of treatment: (*a*) closely similar species are described on the same host, *e. g.*, *Septoria lactucae* and *S. consimilis* on *Lactuca sativa;* (*b*) more widely variant forms on distantly related hosts are described as representing one species, *e. g.*, *S. graminum* on twelve or more genera of grasses, and a species of sedge. It is obvious that such practice is not consistent, and cannot adequately reflect the truth.

The facts as presented above are illustrative of the present unsatisfactory status of the species not only of Septoria but also of many other genera of the fungi imperfecti. More accurate knowledge regarding host characters, morphological variation, and biological specialization is greatly needed from the standpoint of classification. The genus is the more worthy of study on account of its high economic importance. To study such a large genus as Septoria in culture, either upon the living hosts or upon artificial media, would require the time of many investigators. The present paper is intended merely as a contribution to the general problem as suggested above, and the investigations were conducted with the following leading objects:

1. To determine the host range of as many species of Septoria as possible in order to ascertain (*a*) whether morphologically similar forms from different hosts vary in host range, and (*b*) whether morphologically unlike forms ever have the same host range; or in other words to ascertain whether any of the species available for study consist of a number of biologic forms, and whether any now listed as distinct species are identical.

2. To compare disease characters, *i. e.*, the host response to infection, when produced (*a*) by a single species of Septoria upon dissimilar hosts, or (*b*) by different species of Septoria on the same host.

3. To note any morphological changes in the size and shape of spores and pycnidia as a result of change of host or the condition of the host, or of other environmental factors.

4. To determine the susceptibility of different parts of the same host and of these at different stages of maturity, and to note whether any distinctions in disease characters are correlated with host structure and condition.

Historical Review

The literature dealing with biologic specialization is extensive. A comprehensive review cannot be undertaken here, yet a brief summary of the essential points, especially as brought out by the investigations of rusts and powdery mildews, will be useful in the interpretation of the results to be presented. It may be well to mention that biologic specialization is also spoken of as "adaptive parasitism," and biologic forms are referred to as "biologic species," "biologic races," "physiological races," and "adapted races."

Eriksson (8) called attention definitely to the nature of biologic specialization through the results of his cross-inoculation experiments with *Puccinia graminis* in Sweden. He found that, although this rust upon the cereals and grasses represented the same morphological species, the form upon one host-species was not always identical with the form upon another; since, for example, oat rust would transfer to oats, but not as a rule to other species of Gramineae. He showed that this fungus embraces at least six distinct forms distinguished by their dissimilar powers of infection with respect to the species of the grass family. In a subsequent paper (9) Eriksson showed that the trend of specialization may be different in isolated localities. This was illustrated by the fact that the form on rye, *Puccinia graminis secalis*, has a relatively vigorous development in Sweden, but a relatively weak one in North America. In the case of *Puccinia graminis tritici* on wheat, the more vigorous development is in the latter country.

Ward (46), experimenting with *Puccinia dispersa* upon Bromus, discovered "bridging species." The nature of a "bridging species" is described by this statement of the author: "Although it is generally true that the adapted races of *Puccinia dispersa* are restricted to groups of closely allied species, there do occur species which serve as intermediaries in the passage of the fungus from one section of the genus to another."

Stakman and Piemeisel (43) claim that the biologic forms of *P. graminis* can be distinguished from one another morphologically as well as biologically, by the size, shape, and color of the urediniospores. Regarding susceptibility they state: "All gradations in susceptibility occur from complete immunity to complete susceptibility to the various biologic forms. The following reactions may be made to inoculation: no visible effect, appearance of small flecks, production of very small uredinia without flecks, production of very small uredinia in small or large flecks, production of large uredinia surrounded by small dead areas or by apparently healthy tissue."

Arthur (1), Hitchcock and Carleton (13), Carleton (2, 3), and Stakman (42) have also carried out valuable investigations upon biologic specialization in rusts.

Neger (21) and Marchal (17) were early workers upon adaptive parasitism in the powdery mildews. Salmon, however, has done the most extensive work in this field, and has published a long series of papers (30–39). He proved that several biologic forms of Oidium parasitize the species of Bromus, that an individual species may be the meeting place of several biologic forms, and that "bridging species" exist. An additional discovery is best stated in his own words (35, p. 57): "The inter-relations of the biologic forms with certain of their host-plants become complicated by the existence of 'biologic forms' of the host-plants." Salmon found that in *Bromus mollis* there is a susceptible and an immune race with respect to four separate biologic forms of the mildew.

G. M. Reed (24–29) has corroborated the results of Salmon and others, and has carried out extensive cross-inoculation experiments with *Erysiphe graminis* (27, 28, 29) in which he has shown considerable variation in susceptibility among the species and varieties of Triticum, Hordeum, Avena, and Secale, a few of which are immune. In *Erysiphe cichoracearum* upon Cucurbitaceae (25) he found but one biologic form, capable of growing upon various members of this host family.

The more important instances of biologic specialization reported in other groups of fungi are as follows: by Stäger in Claviceps (41), by Diedicke in Pleospora and its conidial stage Helminthosporium (5), by Gilbert in *Plowrightia morbosa* (11), by Müller in *Rhytisma acerinum* (20), and by Hesler in *Sphaeropsis malorum* (12). Shear and Wood (40) found that the races of *Glomerella cingulata* from different hosts vary somewhat in the vigor of their attack upon other hosts. Tests by Westerdijk (47) indicate the absence of biologic specialization among similar races of *Sclerotinia libertiana*. Rands (23) has proved by cross-inoculation that the Alternaria of Datura and that of potato are not identical as some have supposed.

With species of Septoria only a few investigators have worked. Levin (15) inoculated several plants akin to the tomato with *Septoria lycopersici*. Definite small black spots without pycnidia appeared on potato, but no effect was seen on other plants. Norton (22) performed similar experiments in humid inclosures, and obtained infection upon several species of Solanum. A further discussion of this author's results will be given in a subsequent section. Stone (45) proved by infection that *Septoria ribes* and likewise its perfect stage *Mycosphaerella grossulariae* taken from *Ribes nigrum* will infect *R. grossularia*, *R. rubrum*, and *R. oxyacanthoides*.

Montemartini (18) has concluded that parasitic fungi are extremely sensitive to the chemical composition of the nutritive medium on which they live, and that under its influence they acquire characters of adaptation, which attain to a certain fixity. In consequence fungi may become unable to flourish on species different from those to which they have accustomed themselves, or even on other portions of the same plant which they inhabit, or in different developmental stages of such plant or organ. This problem is further complicated by atmospheric conditions, and the influence thereof upon sensitivity to attack as well as upon the virulence of the infecting bodies.

The review here given serves to illustrate the intricate nature of biologic specialization, and to remind one of the various factors to be considered in the interpretation of the results of inoculation experiments.

Experimental Methods and Material

The species of Septoria for investigation were with few exceptions collected in the vicinity of Urbana, Illinois. During the growing season spore-bearing material was gathered in the field from naturally infected plants. In fall and winter spores were obtained in most cases from pure cultures isolated before the first frost. In preparation for inoculation, spore suspensions were made with distilled water and were concentrated enough to contain fifty to one hundred spores per loop.

The experiments were begun in June and were continued to the following April. In summer the work was conducted in the field upon plants growing in their natural state. Freedom from disturbance and as far as possible from natural infection governed the selection of suitable individuals. After the first frost the experiments were continued in the greenhouse upon potted plants transplanted from nature or grown from seed.

The method of inoculation was somewhat varied. In the field an atomizer was most convenient, for the spore suspension could be carried safely in the glass container. In the greenhouse a wire loop was more commonly used to spread drops of the suspension over the leaf surface. Where a bloom or other epidermal structures made it difficult to secure good contact, especially with leaves of grasses, the suspension was rubbed on with the clean finger tips with proper care. Consistency of method was followed in a single series to secure comparable results. The entire surface of certain leaves was inoculated, while adjacent leaves of the same plant or of near-by plants were used as checks to detect foreign infection where there was danger of this. Inoculations were made upon individuals of the original host to afford a check upon the viability of spores, or upon unfavorable environmental conditions.

In providing conditions favorable for infection, covers made of paraffined paper bags were found to give the most satisfactory results. Some use was also made of bell jars. The inoculated leaves, or the entire plant, were covered for periods varying from three to five days. In cases in which disease spots without pycnidia resulted, the bags were replaced over the plants for a second period to promote the growth of the fungus, and to induce if possible the development of spore-bearing bodies. If this failed, the spotted leaves were detached and put into a sterile moist chamber. In this manner pycnidia were obtained and infection proved in some cases in which results would otherwise have been in doubt. This was especially necessary in instances in which spore suspensions were made from natural material, and the spores of other fungi might have been present to cause the spots.

In the following accounts the results of the inter-inoculations with each species of Septoria are presented graphically by means of diagrams, and in more detail in a few representative cases by means of tables. The arrows in the diagrams indicate infection, and the lines ending in bars non-infection.

The denominators of the adjacent fractions state the total number of leaves inoculated, and the numerators the number of leaves having one or more infected spots. The total number of spots formed is given in the tables where this is required for a closer comparison of susceptibility. In the second column of each table the letter *f* indicates that the experiment was conducted in the field or out of doors, and *g* that it was carried on in the greenhouse. The letter *b* with the accompanying figure records the number of days the plant was bagged immediately following inoculation.

The lists of hosts given were compiled partly from Saccardo's "Sylloge Fungorum," and partly from data furnished by the herbaria of the New York Botanical Garden and the United States Department of Agriculture.

SEPTORIA POLYGONORUM DESM.

All collections of Septoria made from species of Polygonum at Urbana [illegible]re determined as *Septoria polygonorum* Desm. This fungus has been [illegible]rted upon the following species of Polygonum, which are arranged in [illegible] [illegible]

[illegible]ose [illegible]fected by the author

P. persicaria
" *pennsylvanicum*
" *lapathifolium*
" *orientale*

B. Inoculated but not infected

P. amphibium
" *hydropiper*
" *convolvulus*

C. Not used in experiments

P. bistorta
" *sieboldii*
" *mitis*
" *hydropiperoides*
" *muhlenbergii*
" *dumetorum*

D. Synonymous

P. incarnatum = *P. lapathifolium*
" *nodosum* = *P. lapathifolium*

The members of group *C* are foreign, or were not found growing in this vicinity. The species of group *B*, as well as others that gave negative results when inoculated, apparently had sufficient opportunity to become infected in nature; for infected plants of smartweed were usually growing in close proximity to the uninfected kinds. Yet repeated search for material resulted in finding *Septoria polygonorum* upon only *Polygonum persicaria*, *P. pennsylvanicum*, and *P. lapathifolium*, upon which the form in this locality appears to be specialized. The plants of *P. orientale* were few and apparently escaped infection by being isolated. It remains an open question whether the forms of *Septoria polygonorum* reported upon *P. amphibium*, *P. hydropiper*, and *P. convolvulus* of group *B* are differently specialized or whether the hosts are resistant in this region. The former case is likely if the fungi and their hosts were correctly determined.

TABLE I

Infections with Septoria polygonorum Desm.

Date	Conditions	Plants Inoculated	No. of Leaves Infected and Inoculated	No. of Spots	Check Infections on Original Host
		Spores from *Polygonum pennsylvanicum*			
June 28	f-b 2	*Polygonum persicaria*	12/16	many	8/10
Aug. 30	f-b 2	" "	11/20	"	
Sept. 6	f-b 3	" "	8/12	45	
Sept. 14	f-b 3	" "	10/15	26	
			41/63		
Aug. 30	f-b 4	*Polygonum lapathifolium*	3/10	34	6/6
Sept. 14	f-b 4	" "	5/5	many	6/6
			8/15		
Sept. 14	f-b 4	*Polygonum orientale*	6/6	28	6/6
June 9	shaded	*Polygonum erectum*	0/10		4/5
June 28	f-b 3	" "	0/11		8/[illegible]
			0/21		[illegible]
June 28	f-b 0	*Polygonum convolvulus*	0/8	[illegible]	[illegible]
June 28	shaded	*Polygonum hydropiper*	0/45		[illegible]
Sept. 7	shaded	*Polygonum acre*	0/12		
Sept. 21	f-b 4	" "	0/20		plus
			0/32		
Sept. 21	shaded	*Polygonum scandens*	0/25		plus
June 28	shaded	*Polygonum amphibium*	0/7		8/10
		Spores from *Polygonum persicaria*			
July 10	shaded	*Polygonum pennsylvanicum*	0/10		10/15
Oct. 3	g-b 5	" "	5/5	many	
			5/15		
Sept. 9	f-b 4	*Polygonum lapathifolium*	7/10	many	10/10
Sept. 20	f-b 4	*Polygonum orientale*	7/7	many	
July 10	shaded	*Polygonum convolvulus*	0/20		10/15
Mar. 3	g-b 3	" "	0/5		
			0/25		
July 10	shaded	*Polygonum hydropiper*	0/30		10/15
Sept. 28	f-b 4	" "	0/25		plus
			0/55		
July 10	f-b 3	*Polygonum erectum*	0/25		10/15
Sept. 7	shaded	*Polygonum acre*	0/10		
Sept. 21	f-b 4	" "	0/20		
			0/30		
		Spores from *Polygonum lapathifolium*			
Sept. 11	f-b 3	*Polygonum pennsylvanicum*	2/5	15	
Sept. 21	f-b 3	*Polygonum persicaria*	6/12	15	8/8
Mar. 10	g-b 3	" "	3/4	13	
			9/16	28	

TABLE I—*Continued*

Date	Conditions	Plants Inoculated	No. of Leaves Infected and Inoculated	No. of Spots	Check Infections on Original Host
		Spores from *Polygonum lapathifolium*			
Sept. 21	f-b 3	*Polygonum orientale*	7/7	many	8/8
Sept. 21	f-b 4	*Polygonum hydropiper*	0/25		8/8
Sept. 21	f-b 4	*Polygonum acre*	0/20		8/8
Sept. 21	shaded	*Polygonum scandens*	0/20		8/8
Oct. 7	g-b 4	*Polygonum convolvulus*	0/12		
Dec. 3	moist chamber	*Polygonum convolvulus* (leaves detached)	3/7	5	
Oct. 7	g-b 4	*Fagopyrum esculentum*	0/10		
Dec. 3	moist chamber	*Fagopyrum esculentum* (leaves detached)	0/7		
Dec. 3	moist chamber	*Rumex crispus* (leaves detached)	0/7		

Septoria polygonorum was isolated, and the growth characters were alike in the fungus as isolated from each of the four hosts in group *A*. No distinctions in biological characters, as shown by their powers to infect, were apparent among these four stocks of the same Septoria from as many different hosts, at least in the light of the experiments thus far conducted.

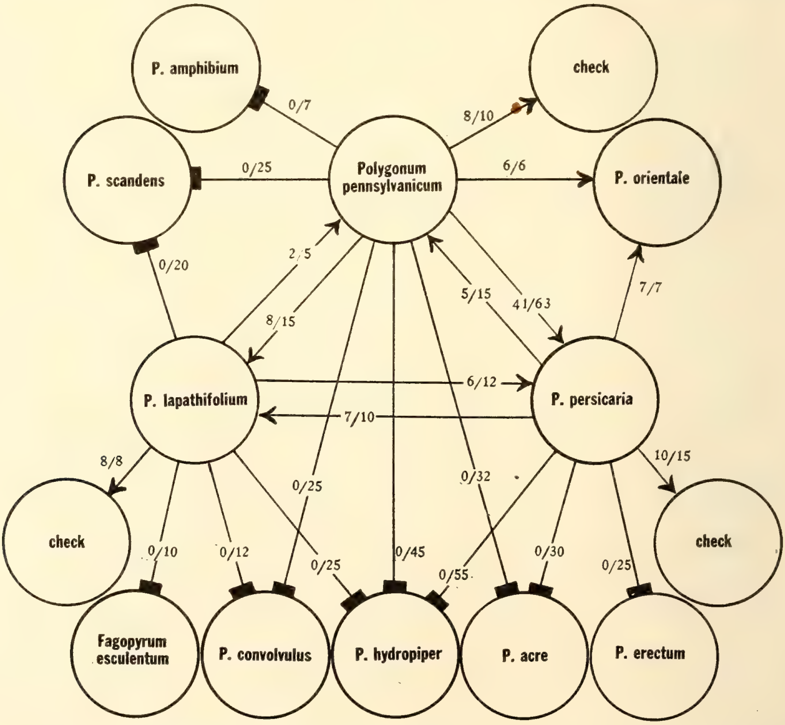
P. amphibium
check
0/7
8/10
P. scandens
0/25
Polygonum pennsylvanicum
6/6
P. orientale
0/20
2/5
5/15
41/63
7/7
8/15
P. lapathifolium
6/12
P. persicaria
7/10
8/8
0/25
0/32
10/15
check
0/10
0/12
0/25
0/45
0/55
0/30
0/25
check
Fagopyrum esculentum
P. convolvulus
P. hydropiper
P. acre
P. erectum

DIAGRAM I. Infections with *Septoria polygonorum* from *Polygonum pennsylvanicum*, *P. persicaria*, and *P. lapathifolium*.

Although a vigorous parasite upon the few species of Polygonum, the form of *Septoria polygonorum* dealt with here is not at all cosmopolitan in its parasitism. A special attempt was made to infect a plant of *P. convolvulus* kept constantly covered with a paraffined paper bag to insure a humid atmosphere and provide partial shading, but with negative results. One hundred leaves of *P. hydropiper* also gave negative results. The fungus was induced to attack detached leaves of *P. convolvulus* kept in a moist chamber in the moist laboratory, spots and pycnidia developing in seven days; but it would not attack *Rumex crispus* or *Fagopyrum esculentum* in a similar way. Thus *Septoria polygonorum* appears as a species with fairly fixed infection-powers, possibly embracing forms differently specialized in addition to the one experimented with here.

The disease spots are not the same on the various species of Polygonum. The distinctions are illustrated in Plate I, figures 1–4. The specimens were selected as representing the usual character of the spots. Specimens of the same species might have been selected showing scarcely distinguishable spots. Upon *P. pennsylvanicum* the spots attain the greatest size, often become irregular in outline, have but a very narrow, dark brown border, and have pycnidia uniformly distributed throughout their area (fig. 1). The spots upon *P. persicaria* are intermediate in size, and are chiefly characterized by a rather wide, dark reddish-brown border. The spots seldom lose their orbicular shape, and the pycnidia are grouped nearer the center (fig. 2). Upon *P. lapathifolium* the spots range smallest in size, and are yellowish-brown in the fresh leaves, with a border of the same color, but in the dry specimens the spots become reddish-brown (fig. 3). In size and shape the spots on *P. orientale* resemble those on *P. pennsylvanicum*, but are yellowish-brown in the fresh leaf. The reddish-brown border appeared when the leaf was put into a moist chamber for a day. The variability in the biological or host characters shown here illustrates the untrustworthy nature of these characters when used to distinguish species.

SEPTORIA LACTUCICOLA ELL. & MART.

Septoria lactucicola has been reported upon *Lactuca floridana*, *L. scariola*, and *L. canadensis*, but in most instances on the last-named plant. The results in diagram 2 show that this Septoria is somewhat adaptive in its parasitism, being able to attack three genera and five species. Were the experiments conducted on a broader scale, the host range would probably be enlarged. There is a gradation in susceptibility passing from *L. canadensis* through to *Sonchus oleraceus*. Although the fungus attacked *L. sativa* and *L. scariola* rather readily, with 16 spots on 30 leaves and 10 spots on 35 leaves respectively, the infection was slight compared with 23 spots on 7 leaves of the original host. Moreover, there were but few fruiting bodies on these new hosts. The most of the 17 spots on *Prenanthes* sp. were very small, and *Sonchus oleraceus* was a very uncongenial plant for the fungus. The comparison will be more clear with a description of the spots.

Septoria lactucicola produces upon *L. canadensis* round, reddish-brown to black spots, usually from 3 to 15 mm. in diameter, and frequently with concentric zones of lighter and darker color. *L. scariola* was the only new host in which these characters were preserved, and here the spots were lighter-colored, probably on account of the thinner leaves. Upon *L. sativa* the spots were spreading, with an indefinite border, and several times larger than those on *L. canadensis* where the border is very definite. They were colored light brown in the center, gradually changing to yellow and then to the green of the normal tissue. No pycnidia were observed in the greenhouse, but they developed in two days upon leaves detached and put into a moist chamber. For a further comparison of the spots on these first three hosts compare Plate I, figures 7–9. The spots upon *Prenanthes* sp. were 1 mm. to 3 mm. in diameter, dark brown, more or less angular, and usually surrounded by a greenish-yellow zone. Pycnidia were seen upon the plant, but spores developed only in the moist chamber. The spots upon *Sonchus oleraceus* were 1 mm. to 5 mm. in diameter, grayish brown, and more or less limited by the leaf veins. A small number of pycnidia with spores characteristic of *Septoria lactucicola* were obtained upon two of several infected leaves placed in a moist chamber.

That the biologic characters change with the host is clearly shown. Such variation has doubtless led to erroneous determinations, or to the useless multiplication of species in many instances. Environment may likewise cause alterations of these characters, for the spots on *L. canadensis* tended to lose their concentric zones and become spreading if kept long in a moist atmosphere.

Septoria lactucae Pass.

The following hosts are reported for *Septoria lactucae: Lactuca sativa*, *L. scariola*, *L. virosa*, *L. canadensis*, and *Chrondrilla muralis*. The fungus appears to cross readily between *L. sativa* and *L. scariola*, and causes upon both of these hosts marked disease. The passage of the fungus from either of these hosts to *L. canadensis* apparently takes place only under very favorable conditions of heat and moisture, for it was necessary to cover the plants for a prolonged time with bags, and to atomize them frequently with water to secure infection. A considerable degree of infection was secured in this way, yet it is probable that this Septoria does not occur commonly on *L. canadensis* in nature. The disease characters on *L. canadensis* were different from those seen upon *L. sativa* or *L. scariola;* the spots were smaller, had no tendency to spread, were darker in color, and commonly had a yellow zone for a border. They were angular in outline, usually 1 mm. to 5 mm. broad, and the central area was brown to black. Pycnidia and spores were present.

Especially favorable conditions were also provided in order to infect *Sonchus asper;* still the transfer of the fungus to this more distantly related host was not difficult, for almost 25 percent of the inoculated leaves were more or less diseased. Such infection raises a question of identity between *Septoria lactucae* and certain of the species of Septoria described upon the genus Sonchus. An examination of exsiccati showed a close similarity between *S. lactucae* and *S. sonchifolia*, but more extensive data is needed to prove their identity. The spots formed upon *Sonchus asper* by *S. lactucae* were grayish-brown to black, angular in outline, 1 mm. to 5 mm. in width, and contained reproductive bodies.

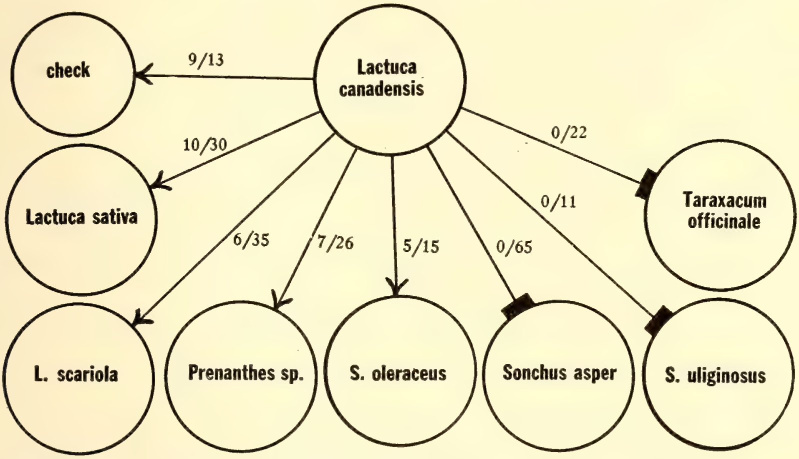
Lactuca canadensis
check
9/13
Lactuca sativa
10/30
L. scariola
6/35
Prenanthes sp.
7/26
S. oleraceus
5/15
Sonchus asper
0/65
S. uliginosus
0/11
Taraxacum officinale
0/22

DIAGRAM 2. Infections with *Septoria lactucicola* from *Lactuca canadensis.*

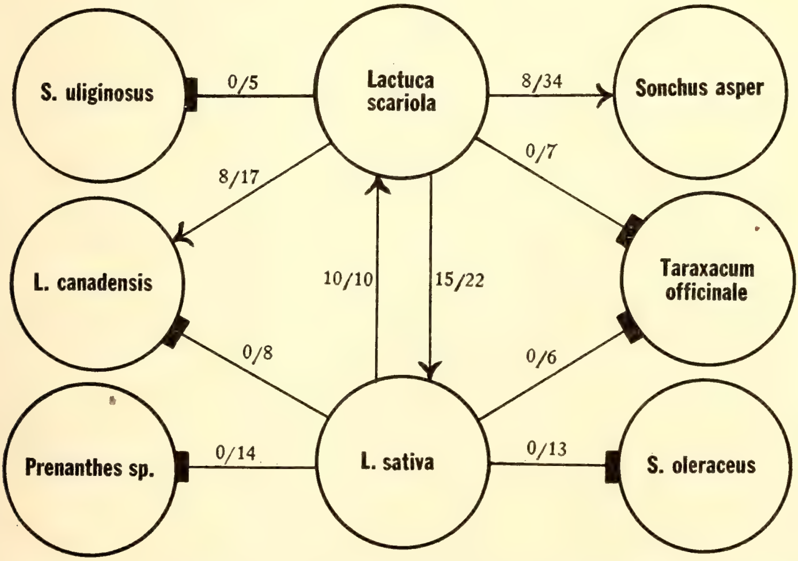
S. uliginosus
0/5
Lactuca scariola
8/34
Sonchus asper
8/17
0/7
L. canadensis
10/10
15/22
Taraxacum officinale
0/8
0/6
Prenanthes sp.
0/14
L. sativa
0/13
S. oleraceus

DIAGRAM 3. Infections with *Septoria lactucae* from *Lactuca sativa* and *Lactuca scariola.*

If *Septoria lactucae* is compared with *S. lactucicola*, it is found that the two species are different in their adaptation to their hosts as well as different morphologically, although the host ranges of the two almost coincide. Each infects *Lactuca sativa*, *L. scariola*, *L. canadensis*, and can pass over to the genus Sonchus. Yet *Septoria lactucae* is best adapted to *Lactuca sativa* and *L. scariola*, grows less readily upon *L. canadensis*, and infects *Sonchus asper* somewhat, while *Septoria lactucicola* is best adapted to *Lactuca canadensis*, grows less readily upon *L. sativa* and *L. scariola*, and infects *Sonchus oleraceus* slightly.

SEPTORIA TRITICI DESM.

There was some uncertainty regarding the name of the species of Septoria from wheat used in these experiments. In the literature two species are frequently mentioned as associated on *Triticum vulgare:* viz., *Septoria tritici* Desm., described in 1842, and *S. graminum* Desm., described in 1843. To the latter is usually ascribed the damage done to winter wheat and other cereals by Septoria. Cavara (4, p. 41) in studying the forms of Septoria on wheat was confronted by so much disagreement that he revised the descriptions of Desmazières as follows:

"Die *Septoria tritici* Desm. (Pl. Cryptog. no. 669), welche anfangs gelbe, dann rostbraune, und endlich weissliche Flecke durch die Zerstörung des Parenchyms bildet, hat gewöhnlich gefächerte Sporen von 50 bis 60 x 1.5–2 μ; sie sind von fadenförmiger Gestalt mit häufig ein wenig aufgetriebener Mitte. Die Scheidewände sind von Desmazières nicht bemerkt worden. Die *Septoria graminum* Desm. (Pl. Cryptog. no. 728) besitzt Perithecien die dem blossen Auge nicht wahrnehmbar und kleiner und dichter gestellt als bei der vorigen Art sind; sie bilden durch ihre Vereinigung längliche grau nebelige Flecke. Die Sporen sind etwas feiner als bei *Septoria tritici* und ein Ende ist dicker als das andere; sie messen 40–50 x 1–1.5 μ, sind nicht gefächert, zeigen aber mehrere Tröpfchen."

Upon the basis of this revision Cavara compared all the exsiccati available to him. His comparisons led him to make this statement (4, p. 22): "Wenn man sich nun Rechenschaft von der grossen Veränderlichkeit der Charaktereigenschaften giebt, wie Form und Farbe der Flecke, Grösse der Perithecien, Vorhandensein oder Fehlen der Scheidewände bei der Gattung Septoria, so wird es sehr wahrscheinlich das *S. graminum* und *tritici* nur Formen einer einzigen mycologischen Art sind und die sich ergebenden Differenzen vielleicht nur der Verschiedenartigkeit der Wirtspflanze zuzuschreiben sind."

The collections of Septoria from wheat used in the present investigation were made from one plot of Turkey Red winter wheat at the Illinois Experiment Station. In June, at a time when the grain had fully headed, nearly all the leaf blades were more or less infected. After harvest, volunteer clumps of wheat came up from heads scattered on the ground, and in

October the leaves in these clumps were badly attacked by Septoria. Collections for purposes of inoculation were made at this time, and also in December and January. After the ground froze the clumps were cut out, allowed to thaw slowly, and were then kept in a closed collecting can in the greenhouse. In a few days the pycnidia were forming spores abundantly.

The spores from this source, collected either in summer or winter, were septate, and the pycnidia were brownish-black, round to elliptical when viewed from above, usually arranged in rows between the leaf veins, and were often visible to the naked eye. The spores from the leaves of the plants that had been frozen and then treated as mentioned were much longer but somewhat more slender than spores from material collected in other ways. Septation was difficult to detect and seemed to be absent in some cases, but proper staining made it apparent in most of the spores. The pycnidia under these same conditions were often more than double their ordinary size, but were unchanged in shape. Infections obtained upon wheat seedlings by inoculation with these longer types of spores always resulted in pycnidia of normal proportions, and in shorter spores with more definite septa, typical of the fungus found on the leaves of wheat in summer or fall. A more comprehensive description of these morphological variations will be given in a subsequent section.

Whether one base his conclusions upon Cavara's revised descriptions, or upon his assertion that *Septoria tritici* and *S. graminum* are probably identical, the fungus at hand must be designated *S. tritici* Desm., in one case on account of morphology, and in the other on account of priority. Yet circumstances indicate that the organism is the same as that often reported as injuring wheat and other cereals and called *S. graminum* (4, 14, 16, 19). It is well to note, however, that all the inoculation experiments, the results of which are summarized in diagram 4, were made with spores taken from the leaves of volunteer wheat collected in late fall or winter.

Seedlings grown in flower pots were used exclusively for inoculation with *Septoria tritici.* Following inoculation, the bags were placed over the pots for three days, but were removed temporarily each day to atomize the leaves with water. On the fifth to the sixth day yellow spots began to appear on the leaves of wheat. These spots enlarged and coalesced until half or more than half of the blades were yellow and drooping. Unless the plants in the greenhouse were covered with bags for a second period or set in a large moist chamber, the yellow blades would dry and no pycnidia would develop. The first pycnidia were observed on the twelfth day, and were distributed in "greenish islands" of tissue, which fact showed that the yellowing of the entire leaf surface was not all due directly to the attack of the Septoria.

That the Septoria of wheat under consideration is limited in its host range to the varieties of *Triticum vulgare* appears conclusive from the results of the infection experiments given in diagram 4. In some instances, especially with the other species of Triticum, and with barley, oats, and rye, there was blanching of the inoculated leaves, but this progressed uniformly backward from the leaf tips and was not due to a parasite. This reaction was also subsequent to the appearance of the irregular yellow spots on the leaves of *Triticum vulgare.* No pycnidia were ever detected upon any plant except upon those in which positive infection is recorded. It is interesting to note that the fungus could not infect hosts closely related to *Triticum vulgare* such as *T. durum*, *T. compactum*, and *T. dicoccum.* If the forms of Septoria upon the species of Gramineae listed below are all morphologically alike, as there is reason to believe, it is evident that *Septoria tritici* consists of more than one, possibly of several, biologic forms. To determine the infection-powers of the form on each of these grasses presents a profitable field for further experiment.

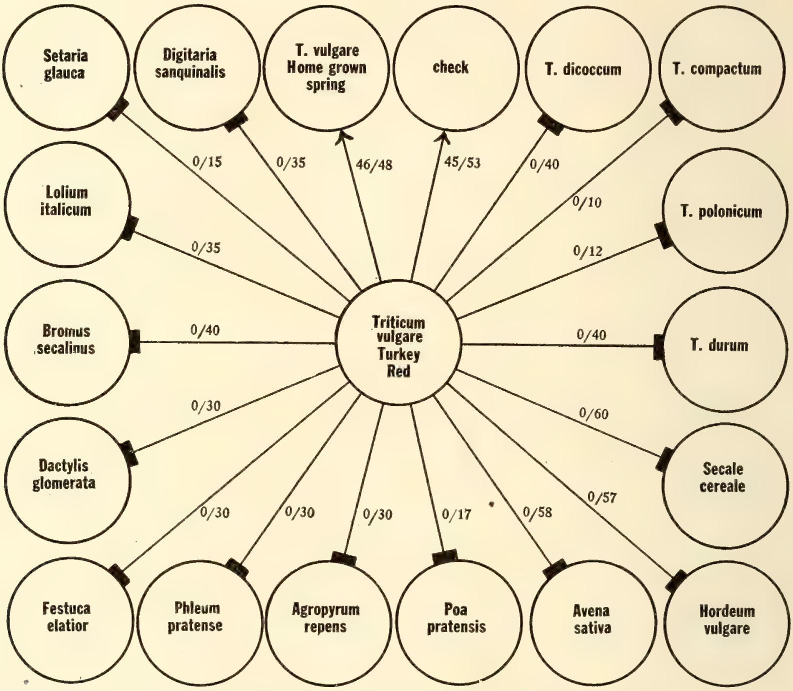
Setaria glauca
Digitaria sanquinalis
T. vulgare Home grown spring
check
T. dicoccum
T. compactum
0/15
0/35
46/48
45/53
0/40
0/10
Lolium italicum
T. polonicum
0/35
0/12
Bromus secalinus
Triticum vulgare Turkey Red
T. durum
0/40
0/40
0/30
0/60
Dactylis glomerata
Secale cereale
0/57
0/30
0/30
0/30
0/17
0/58
Festuca elatior
Phleum pratense
Agropyrum repens
Poa pratensis
Avena sativa
Hordeum vulgare

DIAGRAM 4. Infections with *Septoria tritici* from *Triticum vulgare.*

Hosts reported for Septoria graminum	*Hosts reported for Septoria tritici*
Triticum vulgare	*Triticum vulgare*
Hordeum vulgare	*Triticum caninum*
Avena sativa	*Glyceria fluitans*
Secale cerale	*Brachypodium*
Poa annua	*Festuca*

Poa compressa
Poa pratensis
Bromus sterilis
Calamagrostis epigeios
Calamagrostis langsdorfii
Digitaria sanguinale
Panicum scribnerianum
Paspalum orbiculare
Alopecurus agrestis
Avena planiculmis
Carex riparia

Two series of inoculations were conducted to ascertain whether there was any variation in susceptibility among the varieties of *Triticum vulgare.* In the first series, Turkey Red, Minnesota Reliable, Red Cross, Red Hussar, and Home Grown spring wheat were about equally infected. Pesterboden and Malakoff were infected to a less degree than the above named varieties, while Hungarian was but slightly attacked. In the second series, detailed data were recorded. In terms of percentage, Turkey Red and Beloglina gave each about 52 percent infection if the leaves that had one or more spots of infection were counted. Pesterboden and Malakoff gave 43 percent and Hungarian only 25 percent. This is not a correct basis of comparison, however, for the individual leaves of Turkey Red and Beloglina had the largest and most numerous spots with pycnidia in the greatest number. In Hungarian, often but two or three pycnidia were present on a leaf, while with Pesterboden and Malakoff the condition was intermediate.

SEPTORIA MALVICOLA ELL. & MART.

A Septoria determined as *S. malvicola* Ell. & Mart. was collected upon *Malva rotundifolia* near Hutchinson, Minn. The common mallow is the only host reported for this fungus. The fact that this Septoria gave almost 100 percent infection (diagram 5) when transferred to *Althaea rosea,* the hollyhock, suggests that the fungus may be identical with *Septoria fairmani* E. & E. described on this plant. The identity appears evident when the spot characters produced upon the hollyhock by the mallow Septoria are compared with those of *S. fairmani* as described and as found in exsiccati. The spots on the mallow are smaller and are commonly surrounded by a broad yellow zone. When the fungus was transferred to the hollyhock this yellow zone did not appear, but instead only the narrow black border coincident with the limiting leaf veins was present, features given for *S. fairmani.* When these spots obtained by the inoculation of the hollyhock were compared with *S. fairmani,* North American Fungi no. 3557, there was agreement in all essential points. In the morphology of the fungus the specimens showed no distinctions beyond the limits of variation, and the morphological differences given in the literature are within the range of personal error. The ease with which the fungus passed from the mallow to the hollyhock indicates that this may happen in nature. The plants inoculated in the open were somewhat shaded, and were covered three days with a bell jar. The evidence is sufficient to prove the identity of *S. malvicola* and *S. fairmani*. Leaves of both *Althaea rosea* and *Malva rotundifolia* infected by inoculation with spores from the latter host are illustrated in Plate I, figs. 5 and 6.

SEPTORIA SCROPHULARIAE PECK

Verbascum thapsus was the only host outside the genus Scrophularia to which *Septoria scrophulariae* would transfer, and in this case spots without pycnidia developed. These spots are believed to represent a real cross-infection, since the spores applied were from a pure culture, and the disease was confined to the area inoculated on each leaf. The results of inoculations indicate that *Verbascum blattaria* can be infected to a slight extent, but this was not definitely proved.

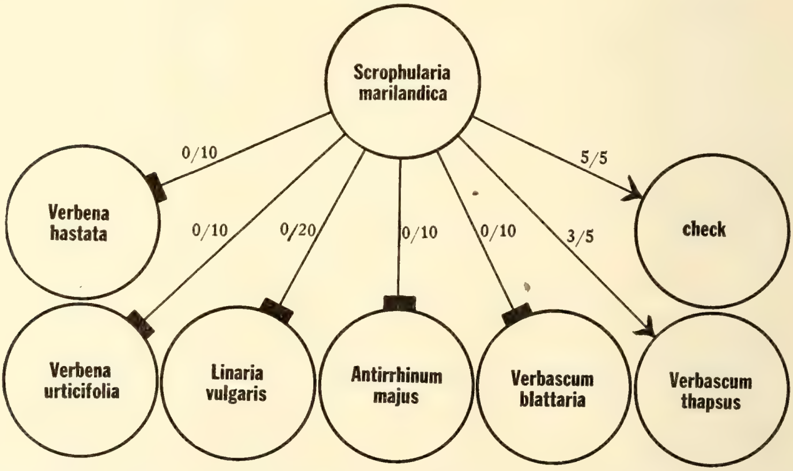

DIAGRAM 6. Infections with *Septoria scrophulariae* from *Scrophularia marilandica*.

A resemblance in many respects between *Septoria scrophulariae* and *S. verbascicola* will be discussed in a following section.

SEPTORIA CONVOLVULI DESM.
SEPTORIA SEPTULATA SP. NOV.

In the Saccardian description of *Septoria convolvuli* Desm., both *Convolvulus arvensis* and *C. sepium* are given as hosts, but the following distinctions in morphology are noted in the forms from the respective hosts:

"In forma *Conv. arvensis* observavi sporulas 35–50 x 1–1.5, aciculares minutissime 5–6 guttulatas v. septulatas hyalinas; in forma *Calystegiae* sporulas 35–40 x 1–1.5, continuas, hyalinas utrinque obtusiusculas. An differentiae constantes?" (Saccardo, Sylloge Fungorum, 3: 536.)

The forms of Septoria collected at Urbana upon *Convolvulus arvensis* and *C. sepium* fitted respectively the characterizations of the above-named forms as quoted. One hundred spores from *C. arvensis* had an average spore length of 44 microns, whereas a like number of spores from *C. sepium* averaged only 35.5 microns. There was a difference in average spore length whatever the conditions under which the two forms were compared. The distinctions mentioned regarding spore tips is not evident, but the spores from *C. arvensis* were always the more definitely septate. With respect to host characters there is little by which the forms can be distinguished.

The results of the infection experiments (tables 2 and 3) proved that the forms of Septoria from these two bindweeds are likewise distinct in their powers of infection, for each can cause vigorous infection only upon its original host. The fungus from *C. arvensis* does infect *C. sepium* to some degree, with 9 out of 39 leaves inoculated showing a few small disease spots, but this is to be compared with 39 out of 45 leaves upon the original host, on which the spots were ordinarily so numerous and so spreading that the leaves were entirely killed. When *C. arvensis* was inoculated with the

TABLE 2

Infections with Septoria septulata sp. nov. from Convolvulus arvensis

Date	Conditions	Plants Inoculated	No. Leaves Infected and Inoculated	No. of Spots	Remarks
Oct. 22	g-b 3	*Convolvulus arvensis*...	15/20	many	Check, most of the leaves killed
Nov. 14	g-b 3	" " ...	4/5	"	
Mar. 2	g-b 3	" " ...	20/20		
			39/45		
June 26	g-b 0	*Convolvulus sepium*.....	3/20	11	Pycnidia and spores but spots very small
Oct. 22	g-b 3	" "	3/3	12	
Nov. 14	g-b 3	" "	2/7	5	
Mar. 18	g-b 4	" "	1/9	1	
			9/39	29	
Nov. 14	g-b 3	*Ipomoea purpurea*......	0/5		
Mar. 2	g-b 3	" "	0/10		
			0/15		
Oct. 22	g-b 3	*Ipomoea batatas*........	0/10		
Nov. 14	g-b 3	" "	0/7		
			0/17		
Mar. 2	g-b 4	*Ipomoea learii*.........	0/10		
Mar. 2	g-b 4	*Ipomoea setosa*.........	0/4		

TABLE 3

Infections with Septoria convolvuli Desm. from Convolvulus sepium

Date	Conditions	Plants Inoculated	No. Leaves Infected and Inoculated	No. of Spots	Remarks
Sept. 10	f-b 4	*Convolvulus sepium*	6/7	21	Check, spots large
Oct. 10	g-b 4	" "	5/5	12	
Nov. 14	g-b 4	" "	5/6	16	
Mar. 18	g-b 4	" "	1/5	4	
			17/23	53	
Sept. 10	f-b 4	*Convolvulus arvensis*	0/7		Few minute spots, no pycnidia, infection doubtful
Sept. 25	f-b 4	" "	0/12		
Nov. 14	g-b 4	" "	0/25		
Mar. 18	g-b 3	" "	0/35		
			0/79		
Nov. 14	g-b 4	*Ipomoea purpurea*	0/5		
Mar. 18	g-b 4	" "	0/5		
			0/10		
Oct. 22	g-b 4	*Ipomoea batatas*	0/5		
Nov. 14	g-b 4	" "	0/10		
			0/15		
Mar. 18	g-b 4	*Ipomoea learii*	0/20		
Mar. 18	g-b 4	*Ipomoea setosa*	0/5		

fungus from *C. sepium*, no disease spots with pycnidia were obtained upon any of 79 leaves inoculated, although minute brown spots, usually less than 1 mm. broad, were often formed. The significance of these spots is unexplained, though they may represent incipient infection.

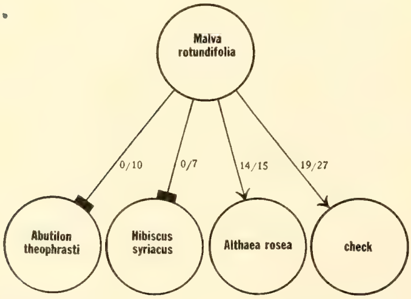
Malva rotundifolia
0/10
0/7
14/15
19/27
Abutilon theophrasti
Hibiscus syriacus
Althaea rosea
check

DIAGRAM 5. Infections with *Septoria malvicola* from *Malva rotundifolia.*

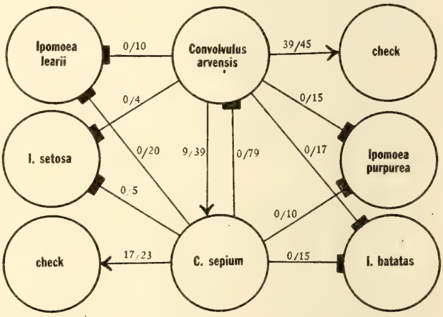
Ipomoea learii
0/10
Convolvulus arvensis
39/45
check
0/4
0/15
I. setosa
0/20
9/39
0/79
0/17
Ipomoea purpurea
0/5
0/10
check
17/23
C. sepium
0/15
I. batatas

DIAGRAM 7. Infections with *Septoria septulata* from *Convolvulus arvensis* and *S. convolvuli* from *C. sepium.*

Septoria convolvuli is reported upon *Ipomoea purpurea*, but all attempts to infect species of Ipomoea were futile.

Were the two forms of Septoria under consideration to be accepted as belonging to a single species, here would be a well established case of biologic specialization. But since the forms have been proved to be different both morphologically and biologically, they should each have specific rank. The name *S. convolvuli* is reserved for the form on *Convolvulus sepium* upon which host the type of *S. convolvuli* was described (6). For the form upon *Convolvulus arvensis* the following new species is proposed:

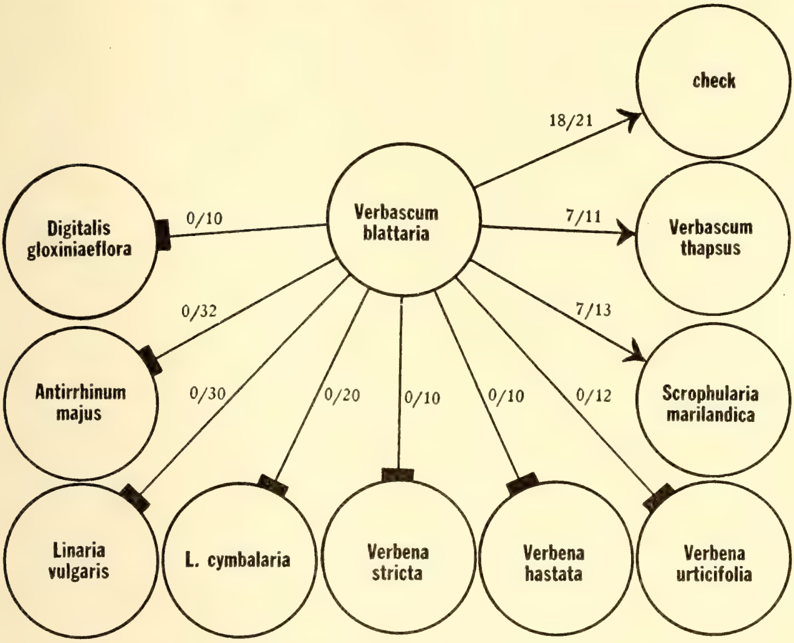
check
18/21
Digitalis gloxiniaeflora
0/10
Verbascum blattaria
7/11
Verbascum thapsus
0/32
7/13
Antirrhinum majus
0/30
0/20
0/10
0/10
0/12
Scrophularia marilandica
Linaria vulgaris
L. cymbalaria
Verbena stricta
Verbena hastata
Verbena urticifolia

DIAGRAM 8. Infections with *Septoria verbascicola* from *Verbascum blattaria.*

Septoria septulata sp. nov.

Spots orbicular, then irregular and confluent, light brown to dark brown; pycnidia mostly epiphyllous, innate, globose, 60–90 μ, protruding with a prominent ostiole, 20–30 μ; spores curved or flexuous, one end narrow with a more acute tip, 3–5 septate, 30–50 μ long by 1–2 μ wide. Habitat: old or fading leaves of *Convolvulus arvensis.*

This species appears to be the equivalent of the form on *C. arvensis* described in Saccardo's Sylloge Fungorum, **3**: 536.

SEPTORIA VERBASCICOLA BERK. & CURT.

Septoria verbascicola appears to be somewhat adaptive in its host relations. The infection of *Scrophularia marilandica* was as vigorous as that on the original host when conditions were made favorable for the fungus. This was done by keeping the inoculated plant constantly covered with a bell jar for ten days, and by atomizing the leaves daily with water following the application of the spores. At the end of this period under cover disease spots began to appear. The plant used seemed to retain its full vigor notwithstanding the rather unusual conditions provided in the experiment. The infection of *Scrophularia marilandica* secured without these special conditions was slight. When the fungus was transferred back to *Verbascum blattaria*, the original host, there was heavy infection, though the inoculated plant was inclosed no more than two days.

The disease spots formed upon *Scrophularia marilandica* were essentially like those upon *Verbascum blattaria.* Upon either host the central area of the spots was ashen in color, while the border was reddish-brown to purplish. Under humid conditions a dull green zone appeared outside the reddish-brown ring, indicating the destruction of new tissue by the encroaching fungus, or the entire area of the spot would be dull green to black. This latter phenomenon was to be seen chiefly upon the original host. Pycnidia were numerous upon both hosts under the humid conditions.

Septoria scrophulariae Peck, which is common upon *Scrophularia marilandica* in nature, forms spots scarcely distinguishable from those above described. The most important distinction is the scanty number of pycnidia; not more than a half dozen are often to be seen in a spot. The spores and pycnidia of *S. verbascicola* and *S. scrophulariae* from material collected near Urbana were so similar that it was suspected that the forms were identical. The fact that the former infected *Scrophularia marilandica* readily made such identity probable, but this view was shown to be questionable when it was found that the latter infected *V. blattaria* slightly if at all. It was discovered, moreover, that the two fungi can be separated easily in culture, as *S. verbascicola* produced pycnidia and spores upon all media used, while *S. scrophulariae* seldom did; the growth of the first was chocolate-colored, that of the second buff.

Septoria verbascicola attacked *Verbascum thapsus* readily, but few spore-bearing structures developed. The spots attained a size as great as those upon *V. blattaria*, were angular in outline, and purplish in appearance. It seems probable that this host is infected in the field, although no proof of this was secured. Leaves of mullein were collected with similar disease spots, some of which were very large, but as no pycnidia were found it cannot be stated that the injury was caused by Septoria.

The rather constant formation of small brownish spots upon the species of Verbena following inoculation with *S. verbascicola* would appear to indicate incipient infection, yet the failure to obtain pycnidia, and to prove that the spots were not due to other causes, leaves the matter in doubt.

Septoria cirsii Niessl.

Upon *Cirsium arvense*, *Septoria cirsii* is an active parasite, both in the field and when brought into the greenhouse. In the field it attacks chiefly the lower leaves and causes large, usually irregular, brown, dry spots, mostly along the leaf margins, but often the whole leaf is involved. In the greenhouse growth is similar, but more rapid and extensive. Inoculated leaves are generally completely destroyed. The fungus is also reported upon *Cirsium discolor.*

According to the infection experiments conducted, *Septoria cirsii* can make a weak attack upon both *Cirsium discolor* and *C. lanceolatum.* The spots formed upon these hosts resemble those upon *C. arvense*, but are smaller and very few in number. The difference in the vigor of attack upon these hosts is also well demonstrated by the length of the incubation period and the rate of development. Upon *Cirsium arvense* spots appeared in thirteen days after inoculation, pycnidia were observed in fifteen days, and in four weeks the whole plant was dead from the spread of the disease. Upon *Cirsium lanceolatum* spots were first visible in twenty days, and in four weeks they had attained a breadth ranging from 3–8 mm. Pycnidia and spores were found at this time. The plant had to be kept in a moist atmosphere to bring about the formation of many spores. The incubation period and rate of development were similar in *Cirsium discolor.* There were no noticeable differences in the morphology of the fungus upon the various hosts.

The relation of maturity of the spores to the presence of septa was often noticed in this species. Where spores were made to develop abundantly in a moist chamber, septa could not be seen at all, or could be seen only faintly after staining with iodine. In old spots, or where the growth had been slow, the septa were plainly seen. Spores obtained from an old culture upon corn meal agar had septa that were strikingly definite. Plants inoculated with spores from this culture were heavily infected.

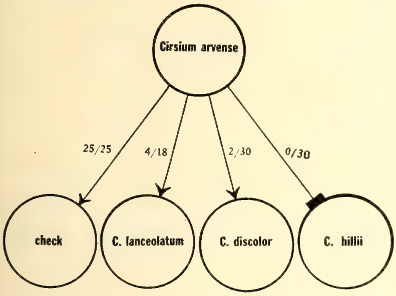

Diagram 9. Infections with *Septoria cirsii* from *Cirsium arvense.*

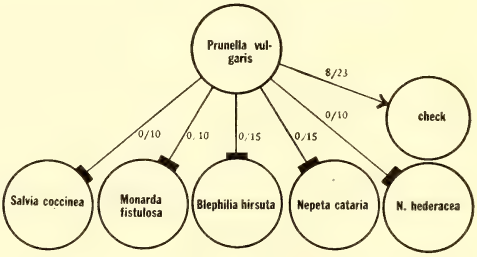

Diagram 10. Infections with *Septoria brunellae* from *Prunella vulgaris.*

Septoria brunellae Ell. & Halst.

The inoculations with *Septoria brunellae* gave negative results in all cases except upon the original host, which facts indicate that the fungus is probably limited to *Prunella vulgaris* in its host range. Upon this one host it is a marked parasite; frequently the whole surface of some leaves is discolored by the confluent spots.

SEPTORIA LYCOPERSICI SPEG.

The inoculations with *Septoria lycopersici* gave results in agreement with those published by Norton (22) who inoculated several plants related to the tomato in humid atmospheres. He states that spots developed better and spores were larger on potato and *Solanum carolinense* than on tomato, while on Datura the spots were slow-growing, light-colored, and small-spored. In the present experiments the spots on potato were darker in color and smaller than those on tomato, while those on *Solanum carolinense* were likewise darker in color, but larger, and often coalesced until the leaves were destroyed. The spots on Datura were very similar to those described by Norton. Spores developed upon the potato and *Solanum carolinense* produced full infection of tomato leaves.

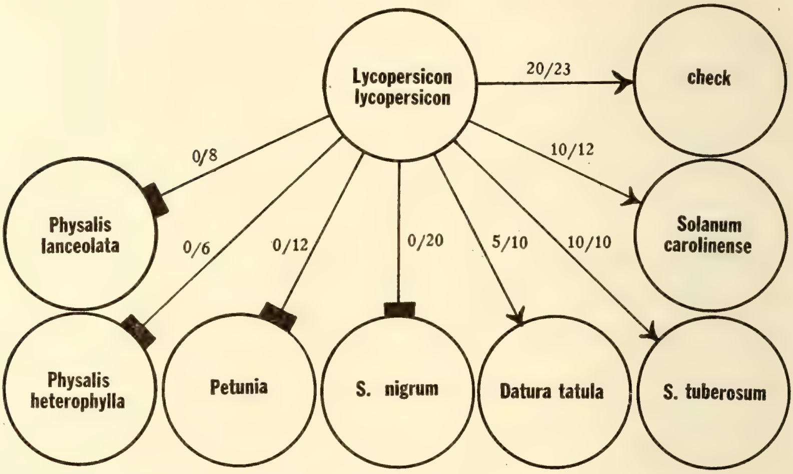

DIAGRAM 11. Infections with *Septoria lycopersici* from tomato.

SEPTORIA LEPIDIICOLA ELL. & MART.

Septoria lepidiicola is reported upon only *Lepidium virginicum* and *L. apetalum*. The data in diagram 12 show that the fungus on these two hosts is identical biologically as well as morphologically. The negative results obtained when plants belonging to other genera of Cruciferae were inoculated indicate that the fungus is confined to the species of Lepidium. No data are at hand to show whether it will attack more than the two species of the genus. The failure to obtain signs of incipient infection, such as spots without spore-bearing bodies, upon species outside the genus Lepidium proves that the fungus has little adaptability. It may be possible, however, to obtain growth in some form upon the remaining species of Lepidium that have not been inoculated experimentally.

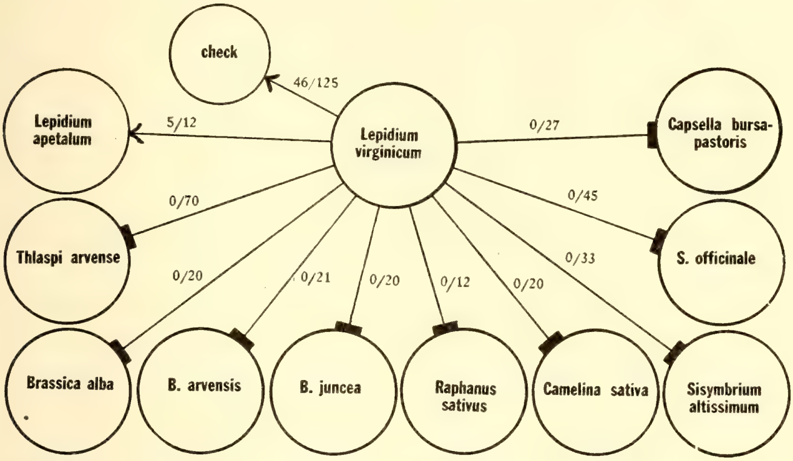
check
46/125
Lepidium apetalum
5/12
Lepidium virginicum
0/27
Capsella bursa-pastoris
0/70
0/45
Thlaspi arvense
S. officinale
0/20
0/21
0/20
0/12
0/20
0/33
Brassica alba
B. arvensis
B. juncea
Raphanus sativus
Camelina sativa
Sisymbrium altissimum

DIAGRAM 12. Infections with *Septoria lepidiicola* from *Lepidium virginicum*.

SEPTORIA HELIANTHI ELL. & KELL.

The following plants have been reported as hosts for *Septoria helianthi:*

1. *Helianthus grosseserratus*
2. " *annuus*
3. " *doronicoides*
4. " *californicus*
5. *Helianthus petiolaris*
6. " *lenticularis*
7. " *strumosus*
8. " *laevis*

In the vicinity of Urbana this Septoria was a common parasite of *H. grosseserratus*, upon the lower or shaded leaves of which it causes grayish-black spots that often become more than a centimeter in breadth. It was less commonly found upon *H. tuberosus*, upon which the spots were smaller, and rarely upon *H. annuus* and *H. rigidus*.

In three separate trials *H. grosseserratus* was readily infected with its own form of Septoria without maintaining a humid atmosphere beyond three days. The other species of Helianthus infected by the Septoria from *H. grosseserratus* are shown in diagram 13. Except for *H. tuberosus*, which grew naturally in the greenhouse yard, all these cross-infections were upon seedlings grown in the greenhouse. Proof of infection was obtained only by detaching spotted leaves and laying them in a moist chamber to induce the development of pycnidia. For this reason, as well as on account of the attack of insect pests, no accurate data could be secured concerning the degree of susceptibility of certain of the hosts, consequently some of the records are wanting. It is clear, however, that the original host is the only one that is congenial for this Septoria from *H. grosseserratus*, for on its own host alone spore-bearing bodies developed naturally under the ordinary greenhouse environment. The infection of small shoots of *H. tuberosus* covered, out of doors, with a bell jar was relatively feeble. Numerous spots developed, but the author could not satisfy himself that they were entirely due to Septoria, on account of the scanty appearance of pycnidia, and because a Phyllosticta, apparently saprophytic however, often appeared when the leaves were placed in a moist chamber.

TABLE 4

Infections with Septoria helianthi Ell. & Kell. from Helianthus grosseserratus

Date	Conditions	Plants Inoculated	No. Leaves Infected and Inoculated	Remarks
Sept. 16	g-b 3	*Helianthus grosseserratus* . . .	5/5	Check, leaves mature, 32 spots.
" "	g-b 3	*Helianthus occidentalis*	0/6	Leaves mature
" "	g-b 3	*Helianthus rigidus*	0/5	" "
" "	g-b 3	*Helianthus mollis*	0/5	" "
" "	g-b 3	*Helianthus tuberosus*	0/5	" "
" "	g-b 3	*Helianthus annuus*	3/8	Seedlings, pycnidia in moist chamber, 10 spots
" "	g-b 3	*Silphium integrifolium*	0/5	
" "	g-b 3	*Bidens cernua*	0/20	
July 5	g-b 3	*Helianthus grosseserratus* . . .	7/7	Check, leaves young, 26 spots
" "	f-b 4	*Helianthus tuberosus*	4/10	Many spots, but few with pycnidia
" "	f-b 4	*Helianthus mollis*	0/7	Leaves young
" "	f-b 4	*Helianthus rigidus*	0/10	" "
" "	f-b 4	*Silphium integrifolium*	0/5	
Jan. 5	g-b 4	*Helianthus rigidus*	plus	Seedlings, only spots formed
" "	g-b 4	*Helianthus argyrophyllus* . . .	4/20	Seedlings, pycnidia in moist chamber
" "	g-b 4	*Helianthus cucumerifolius* .	6/20	As above
Mar. 2	g-b 4	*Helianthus grosseserratus* . . .	plus	Check, seedlings
" "	g-b 4	*Helianthus californicus*	plus	Seedlings, pycnidia in moist chamber
" "	g-b 4	*Helianthus annuus nanus flore pleno*	plus	As above
" "	g-b 4	*Helianthus annuus double primrose queen*	plus	As above
" "	g-b 4	*Helianthus maximilianus* . . .	0/10	
" "	g-b 4	*Coreopsis lanceolata*	0/20	
" "	g-b 4	*Rudbeckia laciniata*	0/10	
		Spores from *Helianthus tuberosus*		
July 24	f-b 4	*Helianthus tuberosus*	4/10	Check, pycnidia and spores
" "	g-b 4	*Helianthus grosseserratus* . . .	0/5	
" "	f-b 4	*Helianthus rigidus*	0/10	
" "	f-b 4	*Helianthus mollis*	0/10	
" "	f-b 4	*Helianthus annuus*	0/5	
" "	f-b 4	*Silphium integrifolium*	0/5	
		Spores from *Helianthus rigidus*		
Sept. 18	f-b 5	*Helianthus grosseserratus* . . .	0/8	No check, but spores germinated well in laboratory tests
" "	f-b 5	*Helianthus tuberosus*	0/5	

It appears significant that the Septoria from *Helianthus rigidus* did not infect *H. grosseserratus* when the plant, inoculated in the field, was bagged five days, and that the form from *H. tuberosus* did not give ready infection of *H. grosseserratus* in the greenhouse. The failure of infection in the series of inoculations made upon mature plants of various species of Helianthus on September 16 (table 4) in the greenhouse, and also on similar hosts in the field in summer, in which spores from *H. grosseserratus* were used, may have been due to the age of the leaves, yet checks on the original host gave positive results. A corresponding set of hosts were inoculated at these same periods with spores from *H. tuberosus* with negative results, but these were not shown by diagram as no checks were used. Still, in these cases the spores germinated well in laboratory tests.

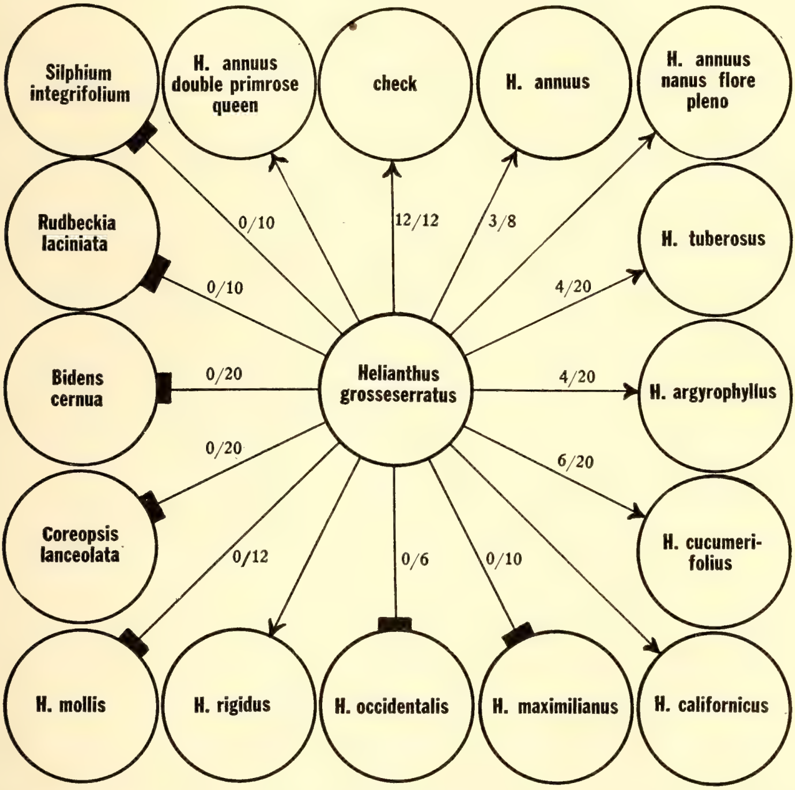
Silphium integrifolium
H. annuus double primrose queen
check
H. annuus
H. annuus nanus flore pleno
Rudbeckia laciniata
H. tuberosus
Bidens cernua
Helianthus grosseserratus
H. argyrophyllus
Coreopsis lanceolata
H. cucumerifolius
H. mollis
H. rigidus
H. occidentalis
H. maximilianus
H. californicus
0/10
12/12
3/8
0/10
4/20
0/20
4/20
0/20
6/20
0/12
0/6
0/10

DIAGRAM 13. Infections with *Septoria helianthi* from *Helianthus grosseserratus.*

All the data obtained respecting *Septoria helianthi* indicate that the forms used in inoculation are not vigorously parasitic except upon their original hosts. These data are scarcely ample for definite conclusions, yet the results are in harmony with what was observed regarding the host range of this Septoria in the vicinity of Urbana. If there are no fixed biologic forms, nevertheless there appears to be a degree of specialization upon the different species of Helianthus, possibly of a temporary nature such as Montemartini (18) claims for rusts.

SEPTORIA RUBI WEST.

Septoria rubi was collected frequently at Urbana upon *Rubus occidentalis*, the black raspberry, but was never found upon any species of Rubus belonging to the blackberry, or Eubatus, section of the genus. Since plants of the blackberry were often growing in close enough proximity to *R. occidentalis* to enable them to become inoculated with the Septoria, it appears that the species of blackberry were not susceptible to the fungus on the black raspberry. During July thirty leaves each of *R. occidentalis* and a species of blackberry were inoculated with spores of Septoria from the former host. Both of these plants inoculated were young, and were growing close together in a shaded place. In two weeks all the thirty leaves of *R. occidentalis* were thickly spotted with Septoria, but no trace of infection could be found upon the blackberry.

No definite conclusions can be drawn from the above-described observations and the accompanying experiment. The facts may indicate that *Septoria rubi* is split into biologic forms, or that the species of blackberry in this locality are permanently resistant to the Septoria. This fungus is reported, however, upon twenty-eight or more species of Rubus, distributed among all sections of the genus, a fact that makes it appear probable that all members of the genus are parasitized. If this be true, the brief data above indicate the existence of biologic forms in *Septoria rubi*. The large number of species in the genus Rubus, of which so many are known to be hosts of Septoria, makes them an attractive field for a further study of biologic specialization.

SEPTORIA ATRO-PURPUREA PECK

The presence of disease spots without pycnidia is the only indication that *Septoria atro-purpurea* from *Aster cordifolius* can infect *A. ericoides* and *A. laevis*. This infection was obtained under prolonged humid conditions, and it is improbable that it occurs often in nature. These asters have not been reported hitherto as hosts for the fungus. This parasite, according to specimens in the Herbarium of the United States Department of Agriculture, attacks *Solidago latifolia* and *Machaeranthera aspera*. The data indicate that this Septoria is able to adapt itself to related hosts to a considerable degree.

The disease spots on *A. cordifolius* are nearly orbicular, with a central area of reddish-brown and a margin of light green. In the larger and older spots a gray area appears within the reddish-brown. Upon *A. ericoides* the spots were a bright reddish color, with a margin of yellow, and a ragged and indefinite outline. The spots on *A. laevis* were very small and brown, indicating that the fungus was less well adapted to this host than to *A. ericoides*. As the spores for inoculation were from pure culture, and adjacent leaves not inoculated showed no symptoms of disease, it appears certain that the spots in question were due to the Septoria applied.

General Discussion

Age incidence. In the field Septoria spots are more frequently observed upon the old or fading leaves of plants, but this is often due to the fact that the young leaves have recently expanded, and have not had time to become infected. The immature foliage is sometimes the more susceptible, but this varies with hosts. From the results of the infection experiments the leaves of *Lepidium virginicum* appear to be equally susceptible at all ages. Old leaves of *Lactuca scariola* in which the edges are cracked and drying are infected without difficulty, but the spots remain small. Partially grown leaves of this host are more easily infected, and the spots become larger. The older leaves of *Convolvulus arvensis* and *C. sepium* are the most heavily attacked, while inoculation of the immature leaves seldom produces infection.

The influence of age upon susceptibility is very well shown in *Malva rotundifolia* and *Althaea rosea.* Disease first develops on the oldest leaves; in those of intermediate age the incubation period has greater length, while those partially grown are resistant. A plant of *Althaea rosea* with seven leaves was thoroughly wetted with a suspension of spores of *Septoria malvicola*, and kept under a bell jar four days. In ten days the five mature leaves were spotted, the oldest having the heaviest infection. It was nearly three weeks before the sixth leaf showed disease, and the seventh or youngest leaf inoculated continued healthy. In two trials with the mallow there were similar results, which are in accord with what one sees in the field, for there only old shaded leaves are badly attacked.

Susceptibility of different leaf surfaces. Equal areas of the upper and the lower surfaces of separate lots of leaves of a number of plants were inoculated, and other factors made as comparable as possible. In *Lactuca scariola* the upper surface gave the heavier infection. In *Polygonum persicaria* 39 spots, 1–5 mm. in diameter, resulted on the top side as compared with six spots, 1–2 mm. in diameter, on the lower. The leaf surface of *Erigeron annuus* in 150 separate areas, 75 above and 75 below, was inoculated with loop-drops of a suspension of spores of *Septoria erigerontis*, and 14 infections from above and six from below were obtained. In contrast to these experiments, the inoculation of the lower surfaces of leaves of *Solanum carolinense* with *Septoria lycopersici* gave abundant infection, while leaves inoculated above remained free of disease. No explanation of the above-described results can now be given, but the number of stomata, the character of the cuticle, the ease with which the suspension makes contact with the surface, and the light exposure may be factors.

Effect of the mass of inoculum. The effect of varying the concentration of the spore suspension was tested. One hundred areas upon *Erigeron annuus* were inoculated with loop-drops containing approximately 100 spores, and an equal number of areas with loop-drops averaging 1–3 spores. In the former case there were 35 areas infected, and in the latter 15, but in the 35 areas over 80 points of infection were noted, while in the 15 areas only 18 were present. Infections were obtained upon *Lactuca scariola* with loop-drops containing only 1–3 spores in 11 percent of the inoculations.

Effect of wounding. Certain leaves of young potted plants of *Malva rotundifolia* were perforated with a fine needle in numerous places. All the leaves of each plant were inoculated over the entire upper surface, and bagged for four days. Only the perforated leaves became infected. Either the wounding had the effect of overcoming the resistance that may have been present in the cell contents of the young foliage, or the pierced epidermis afforded an easy entrance for the germ tubes of the spores.

Variations in the morphology of the fungus. The more detailed studies of morphological variations were made with *Septoria tritici* and *S. verbascicola*, and the chief results obtained are shown in graph i by curves which represent the ranges of spore length in these fungi under a number of different conditions. No investigation was made of the factors causing these variations, but apparently they are due to differences in humidity. An increase in spore length when infected leaves were kept in a moist chamber for a period was apparent in many other species of Septoria.

There was no alteration in spore length when *S. verbascicola* was transferred from *Verbascum blattaria* to *Scrophularia marilandica* under comparable conditions, as *E* and *F* of graph 1 show. Norton (22) reports that the spores of the tomato Septoria become longer upon the potato and *Solanum carolinense*, but shorter upon *Datura tatula* than they were upon the original host, when the inoculations were made within humid inclosures. It is not stated whether he took account of environmental relations other than the change of host, but the lesser spore length upon *Datura tatula* makes it appear that the host has a definite effect on morphology. Still, in the numerous crosses recorded in the tables of the present paper, no changes of spore length were observed for which environmental factors such as humidity might not account, at least in all cases in which the fungus and host were in compatible relations.

If all the species of Septoria have a corresponding relation to the environment, and if all have a broad range between the maximum and minimum spore length, as in *S. tritici* and *S. verbascicola*, it is obvious that many of the measurements now given in specific descriptions are far from accurate. The range of 19 to 62 microns reported for *S. sisymbrii* is not of greater width than that shown for the fungi in graph 1, and indicates care in measurement. It is not proper to compare dry herbarium specimens with material freshly collected, especially if it has been allowed to form spores in a moist chamber. It is well to make a record of the source of the material collected, and the conditions under which it was found. (*Cf.* graph 1, *C*, *D*, *G*, and *H*.)

Host limitations. The experimental results thus far obtained indicate that the species of Septoria do not have a broad host range. Each can infect vigorously one or a few closely allied plants, and can infect to a less degree a number of hosts that stand in rather immediate relation to the vigorously infected ones. In some cases this host range does not extend beyond the limits of a genus, and in other cases includes but two or three related genera. Although some of the fungi studied have been found to have approximately the host range previously reported for them, in many instances the host ranges established by the experiments have been much more narrow than the reports on hosts would lead one to conclude, a few forms being limited to the species upon which they were collected. Notable illustrations of this are the forms of Septoria on wheat, *Convolvulus sepium*, and possibly upon *Rubus occidentalis* and *Helianthus* sp. Further infection experiments would doubtless reveal more cases of identity both morphological and biological among forms now classed as separate species, while at the same time some of the present species would be shown to consist of more than one morphological form, or species.

The value of disease characters. The variable nature of disease characters, as manifested by the host, has been well demonstrated. These variations are dependent upon the species, the age, and the part of the host as well as upon environmental conditions. On this account these characters lose much of their value in taxonomy, but inasmuch as the host ranges of the species of Septoria are not broad, and the number of forms parasitizing a single host is very few, such characters may be of some use in distinguishing the parasites on individual hosts. For these same reasons the host itself will continue to be a valuable key in the determination of the fungus.

Biologic specialization. The experiments herein described have not been broad enough to include forms from all the hosts reported for any species, especially with such fungi as *Septoria rubi* upon numerous members of Rubus, *S. graminum* or *S. tritici* upon several different genera of Gramineae, and *S. polygonorum* upon various species of Polygonum. Great difficulty will inevitably be met in bringing together even a major portion of the respective forms for comparative study, such as would be necessary to establish firmly the existence of biologic specialization, and to ascertain the number of biologic forms. Still, such data as the present investigations furnish indicate clearly that biologic specialization exists in many species of Septoria. This is shown by the fact that in many instances the Septoria from one host either fails to infect, or infects to only a slight degree, certain hosts upon which morphologically similar forms of Septoria are known. In illustration of this, the species of Septoria from wheat, from *Rubus occidentalis*, and from certain species of Helianthus and Polygonum furnish examples.

CONCLUSIONS

1. The results of the present investigations indicate that certain species of Septoria are differentiated into biologic forms.

2. Although some forms show a degree of adaptability in host relations, in general the species studied are limited to one or to a few closely related hosts which they can vigorously infect.

3. In some cases the host range does not extend beyond the limits of a genus, while in other cases two or three related genera are included.

4. In many cases the host ranges established by the experiments have been more narrow than the host indices indicate.

5. Disease characters, as manifested by the host, vary with the host and with environmental conditions, and are therefore unreliable in taxonomy.

6. Certain species of Septoria have been shown to vary considerably in morphological characters under different environmental conditions, and hence the value of measurements now given in specific descriptions is questionable.

7. Inoculation experiments show that *Septoria malvicola* E. & M. and *S. fairmani* E. & E. are identical.

8. Similar experiments show that the form of *S. convolvuli* Desm. described upon *Convolvulus arvensis* is biologically as well as morphologically distinct from the type form of *S. convolvuli* described upon *C. sepium*, and is entitled to specific rank.

The writer takes pleasure in making acknowledgment to Dr. F. L. Stevens, Professor of Plant Pathology, University of Illinois, for helpful suggestions and guidance in the preparation of this thesis. The writer also wishes to express his appreciation of the valuable assistance given by Prof. William Trelease, Head of the Department of Botany, University of Illinois, in the solution of questions of taxonomy. Thanks are due Fred J. Seaver, Curator at the New York Botanical Garden, and Vera K. Charles, Mycologist, U. S. Department of Agriculture, for furnishing data upon the host ranges of various species of Septoria.

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EXPLANATION OF GRAPH 1

This graph represents variations in spore length in *Septoria verbascicola* B. & C. and *S. tritici* Desm. under a number of different conditions. The measurements of spores are indicated in microns by the base line, each space representing one micron. The frequency is indicated on the perpendicular lines, each space representing one spore. The measurements are at intervals of 2.4 microns, and for each curve 200 spores were measured.

A and *B* represent ranges in length of spores from a single culture of *S. verbascicola* upon onion agar; in *A* the spores were from the lower portion of the colony which was moistened by the small amount of water on the agar, while in *B* the spores were from the upper edge of the colony where the agar was drying. *C* represents spores from spots of leaves of *Verbascum blattaria* in the field where the light exposure was intense; *D*, spores from shaded rosette leaves of the same host in the field; *E*, spores from the same host kept under very humid conditions in the greenhouse; *F*, spores of the same fungus growing upon *Scrophularia marilandica*, conditions as in *E*.

G represents the range of spore length of *S. tritici* upon the upper stem leaves of naturally infected wheat plants in the field in July; *H*, spores of the fungus from the same field taken from the basal leaves of volunteer wheat in January. The plants of wheat were dug up and kept for a few days in a closed collecting can in the greenhouse.

EXPLANATION OF PLATE I

Preparatory to photographing, the leaves were treated with hot alcohol to remove the chlorophyll, but this process produced no apparent change in the character of the disease spots. The leaves were then softened in 50 percent glycerine and pressed.

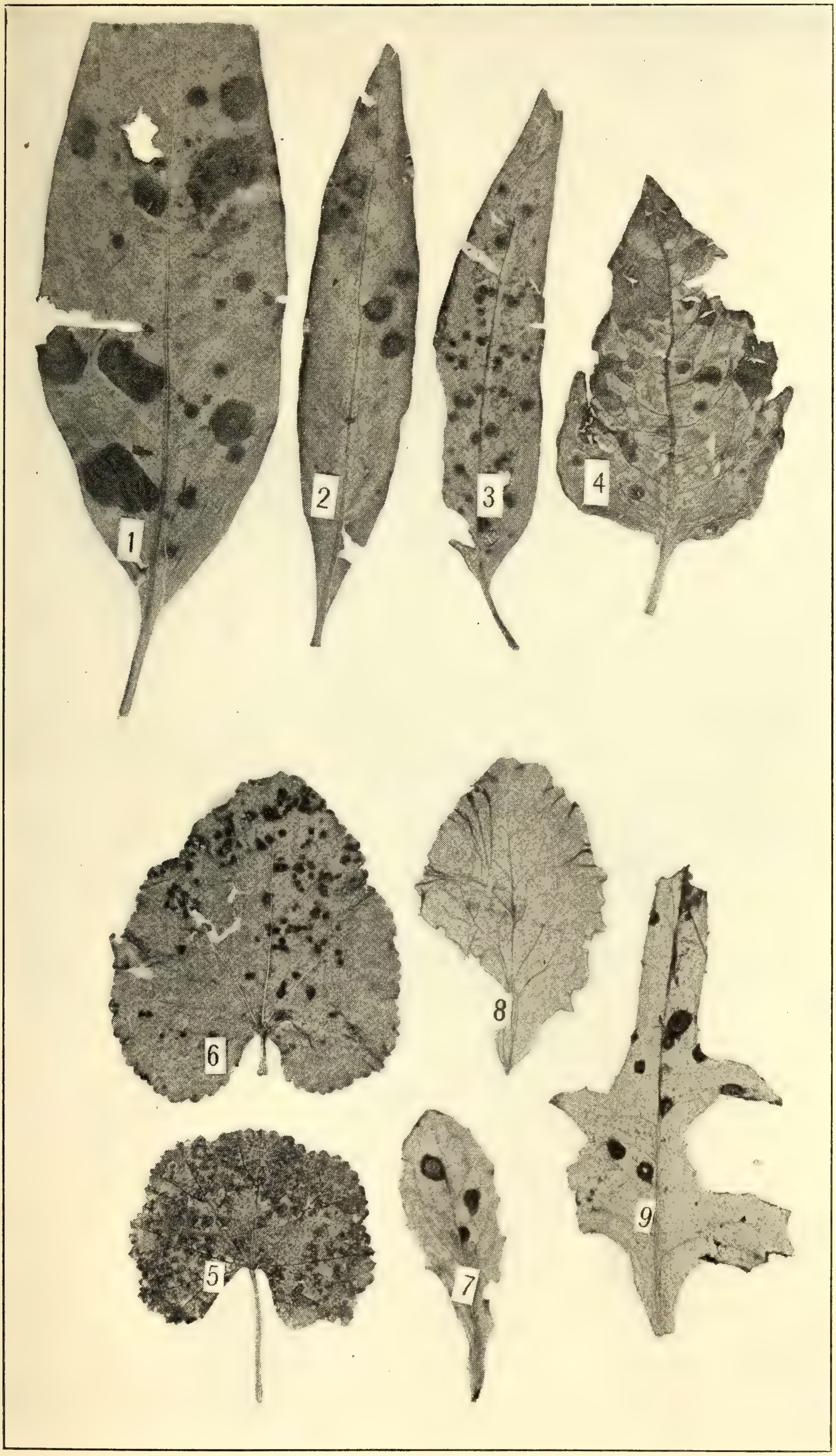
FIG. 1. Disease spots of *Septoria polygonorum* Desm. upon *Polygonum pennsylvanicum*. Natural infection.

FIG. 2. Disease spots of the same fungus upon *P. persicaria*. Natural infection.

FIG. 3. Disease spots of the same fungus upon *P. lapathifolium*. Natural infection.

FIG. 4. Disease spots of the same fungus upon *P. orientale*. Artificial inoculation.

FIG. 5. Disease spots of *Septoria malvicola* E. & M. upon *Malva rotundifolia*. Artificial inoculation.



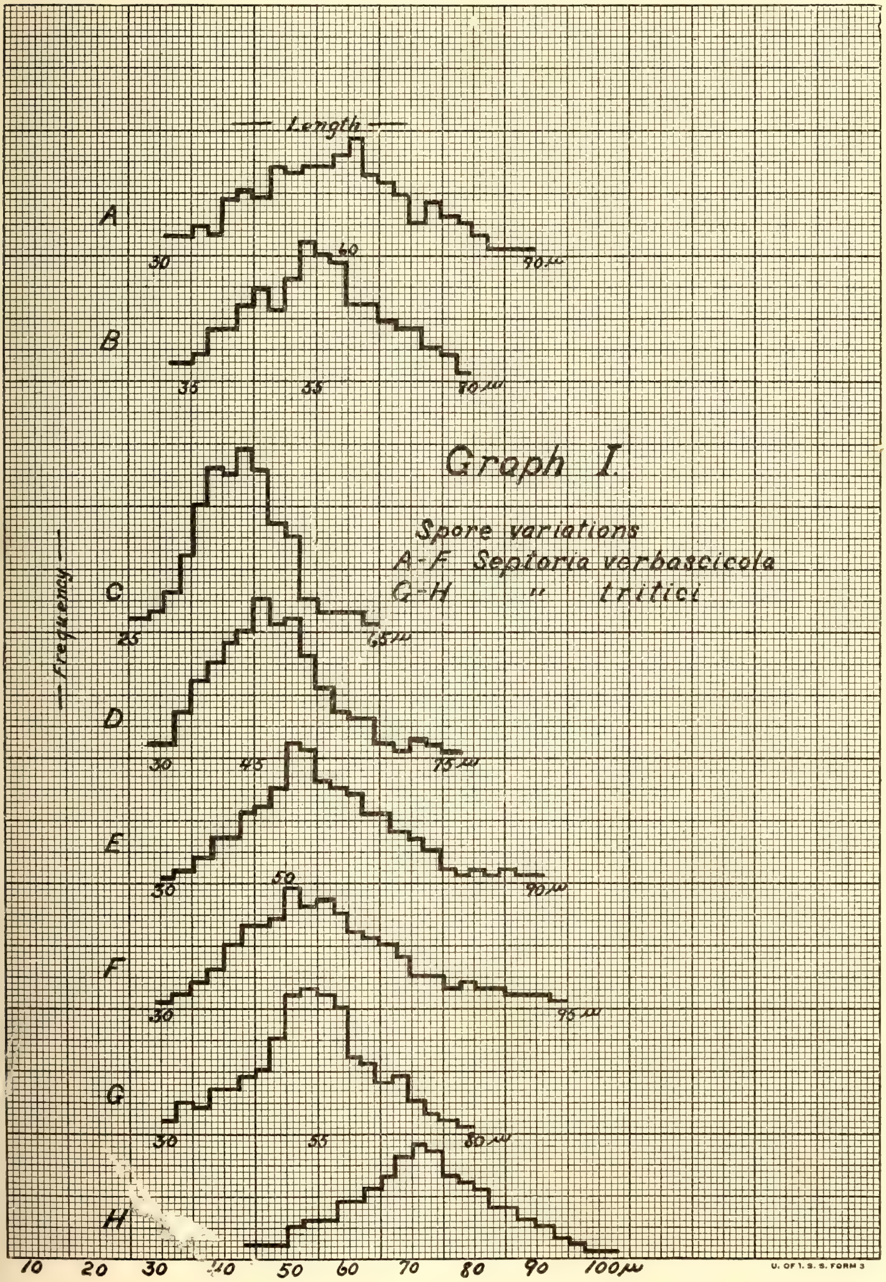
BEACH: SPECIALIZATION IN SEPTORIA.

FIG. 6. Disease spots of *S. malvicola* upon *Althaea rosea.* Inoculated with spores from *Malva rotundifolia.*

FIG. 7. Disease spots of *S. lactucicola* E. & M. upon *Lactuca scariola.* Inoculated with spores from *L. canadensis.*

FIG. 8. Disease spot of *S. lactucicola* upon *Lactuca sativa.* Inoculated with spores from *L. canadensis.*

FIG. 9. Disease spots of *S. lactucicola* upon *Lactuca canadensis.* Natural infection.



A CONSIDERATION OF CERTAIN PATHOLOGIC CONDITIONS IN AMBROSIA TRIFIDA

ALBAN STEWART

In two former publications (8, 9) the writer has shown certain similarities between the structure of gall and that of traumatic tissue. In one of these articles the structure of an insect gall was described and figured; in the other a fungus gall was given similar consideration. The structures involved in both of these galls were compared with the traumatic tissue of the same species of plant on which the galls occurred. Certain similarities in the structure of both of these galls led to some speculation as to what might result anatomically if the same species of plant were attacked by both a fungus and an animal parasite. Furthermore, what would result if both fungus and insect should exert their influence in a gall at the same time, and how would the changes caused by these different stimuli compare with the traumatic tissue of the plant on which the gall occurred?

I have been fortunate enough to secure material which illustrates the effects of these different combinations of stimuli. The stems of the great ragweed, *Ambrosia trifida* L., are sometimes infected by *Protomyces andinus* Lagh., causing large galls. The stems of the same plant are parasitized by *Papaipema nitela* Gn., which causes more or less pronounced swellings on the stem. Furthermore, it is quite often the case that both of these organisms are present in the same gall, and that both have evidently exerted an influence on its structure. Stems which have been wounded and subsequently healed are commonly found along roadsides. A single Protomyces gall was also found which had been wounded. The structure of the pathologic tissues arising from these various causes will be considered in this article.

GALL OF PROTOMYCES ANDINUS ON AMBROSIA TRIFIDA

In a neglected spot near the railroad track at Blue Mounds, Wisconsin, there is a thicket of *Ambrosia trifida* which is badly infected with *Protomyces andinus*. This is the only station in the vicinity of Madison, Wisconsin, where I have found such infections. However, Dr. J. J. Davis, Curator of the Wisconsin University Herbarium, tells me that he has found them rather commonly in other parts of the state. The galls usually occur near the base of the stem just above the ground, and are roughened knot-like, or more or less fusiform swellings, which may involve the entire circumference or only a sector of the stem. In addition to the galls which occur near the ground still other galls may occur higher up on the stem, in some instances two feet or more above the ground. Galls in these various situations are similar both morphologically and histologically so far as has been observed by the writer.

Before entering into the description and consideration of the gall tissue, it seems well to describe briefly the structure of the normal stem. In the cross section of the gall shown in plate II, figure 8, the fungus has confined its activities to one side of the stem, shown on the upper side of the figure, so that only a sector is involved in gall formation. The bundles on the sides and lower part of the figure are entirely normal. In general there are two sorts of bundles in the normal stem: those in which the xylem is broadly triangular or somewhat aquiliform in cross section, and those in which the xylem forms rather narrow radial segments. The narrow bundles are evidently leaf traces, since six of them leave the stem at each node, and according to Sinnott (5) the trilacunar condition is characteristic of many members of the *Compositae*. The leaf trace bundles are formed two nodes below the point where they leave the stem. There seems to be some difference in the structure of the narrow bundles in the young stem, of which two kinds can be distinguished; but as the normal anatomy is to be considered very briefly, it is beyond the scope of this article to enter into a general investigation of the course of the bundles in the stem. The cambium dips in opposite the narrow bundles (fig. 8), resulting in somewhat depressed segments of xylem. It has on this account a somewhat sinuous course around the xylem ring. The broader bundles usually alternate with the narrow ones, but this is not always true, for sometimes two of the broad bundles occur together. Sometimes a ray extends through the center of a broad bundle, cutting it in two. There is often a slight depression in the center of a broad bundle, causing a notch. A bundle of bast fibers is located opposite this notch, shown by the blackened areas in figure 8. The greater part of the phloem is located just inside the bast fibers. The leaf trace bundles are subtended by somewhat larger bundles of bast fibers.

In the young stem the individual bundles are separated from each other by broad rays which extend from node to node. As the stems grow older and the individual bundles approach each other, the rays become narrower, and shorter vertically. Intangential section (fig. 1), the rays are fusiform and are usually three or four cells wide. They are composed of somewhat angular cells which are elongated in a vertical direction. Uniseriate rays are uncommon. With the exception of the ray cells and vessels the xylem is composed mostly of wood fibers. On the outside of the bast fibers the remainder of the bark is composed of several layers of cortical parenchyma cells which are elongated tangentially. There are also several layers of colenchyma cells, just inside the epidermis, which become thickened opposite the trichomes.

A diagram of a cross section of an infected stem is shown in figure 8. The bundles on the upper side of the stem have become greatly altered through the action of the fungus, while those on the lower part and sides have remained normal in structure. In this instance only a sector of the stem has been involved in gall-formation. The xylem on the infected side has been greatly increased both radially and tangentially, while on the other sides it has only its normal growth. Both the leaf traces and the stem bundles can be recognized on the infected side, as the changes brought about by the fungus in this particular instance are less pronounced than in some other specimens examined. Furthermore, the inner extremities of all the infected bundles have their normal structure, showing that this particular part became infected some time after secondary thickening started. Broad intrafascicular rays have been formed, cutting up the bundles into narrow radial segments of xylem. Masses of parenchyma have formed in the xylem where normally there is no parenchyma. There has also been a great reduction in the number of vessels per unit of area, and the size of the vessel has decreased.

The amount of parenchyma, both ray and wood, and the number and size of the vessels vary greatly in different specimens. The specimen shown in figure 8 is about at a minimum in this respect. In extreme cases the xylem portions of the leaf traces are often suppressed or nearly so, resulting in broad ray-like masses of parenchyma. Where leaf traces fail to appear there are deep notches in the xylem ring, their position being shown externally by longitudinal furrows in the surface of the gall.

The formation of depressed xylem segments is not unknown in normal stems of the Compositae. According to Solereder (7), Schenck has observed a furrowed xylem mass in an undetermined member of the order recalling the structure of the Bignoniaceae. "The furrows in this xylem mass are due to the reduced activity on the part of certain longitudinal strips of the cambium which leads to a smaller production of wood and a proportionally greater development of phloem externally."

It is often the case that the individual bundles disappear entirely and in their place there are masses of parenchyma which show but little distinction between wood and bark. Occasional vessels appear in these masses, and irregularly running strands of cambiform cells.

In this connection it might be well to mention certain peculiar strands of tissue which occur under such conditions. Towards the center of many of the galls, in the region of the pith or in close proximity to the protoxylem portions of the bundles, these strands sometimes appear. They consist of whorl-like arrangements of cambiform cells (fig. 5) which enclose sporanges of the fungus, shown by the dark circles in the figure. Structurally they recall strikingly the tumor-strands described and figured by Smith (6) in stems infected with the crown-gall organism, *Pseudomonas tumefaciens*. Short tracheids sometimes accompany these strands, a condition similar to that described by Smith in some of his strands. They usually extend in a vertical direction but not always for the longitudinal section of one of these strands (fig. 4) also shows cross sections of two other strands. It should be noticed that the strand shown in figure 4 encloses relatively large numbers of the sporanges of the fungus, and that where there are large numbers of sporanges the strand is broader. The line of separation between the strand and the surrounding tissue is sharp, as can be seen in the figure. Near the lower right side of figure 4, a single sporange is shown lying just outside the strand, partly surrounded by cambiform cells, similar to those which make up the strand.

The strands are evidently formed in place near the time of infection and are not a product of later growth which has been pushed in from some other source. This is shown by the fact that in the parts of the gall where they occur all the tissue outside them is very abnormal in structure. That infection takes place while the stems are still very young is evident, for in every gall examined some portion or portions of it showed tissue abnormalities in or nearly to the pith, as well as sporanges of the fungus. Furthermore, there is no indication of crushed cells, or of cells which have been partially absorbed along the course of the strands, such as would probably be the case if the strands represented an invasion from some other source. They are evidently caused by some stimulus emanating from the fungus which brings about active and rapid cell division, but a stimulus which is very local in its effects. It seems to be true in general for this gall that the diffusion zone of the gall stimulus, gall poison, or whatever name we wish to apply to the cause of the gall formation, is very limited in extent. Bundles which are very abnormal in structure and which contain the sporanges of the fungus often occur next to bundles which are entirely normal and free from the fungus. Individual bundles sometimes show abnormalities on one side and nothing but normal tissue on the other. It sometimes happens that strands are formed shortly after secondary thickening has started, and in such cases the protoxylem portions of the bundles are often surrounded by a cambium-like zone of cells.

As the strands occur both in the galls near the ground and in those higher up on the stem, one might expect to find them extending through the intervening normal portion of the stem connecting one gall with another—a condition similar to that described by Smith in the crown gall. Such is not the case, however; they are purely local in their occurrence and extend but little above or below where the infection first took place in the stem. (For a possible explanation of the origin of the higher galls see Stewart, 10.)

The bundles show different degrees of disturbance at different levels. At some level or levels in the gall the tissues are abnormal into or beyond the protoxylem of the bundles and probably represent the places where the fungus first began its activities. Going above or below the level of greatest disturbance the xylem becomes less and less involved, until at the upper and lower limits of the gall only that portion which lies just inside the cambium shows structural abnormalities. Somewhat similar conditions are also found in a peripheral direction, as a larger sector of the stem is involved where the tissue disturbances are deepest than at the upper and lower parts of the gall. This fact is well shown in figure 8, where the inner parts of the bundles are normal. The statements just made are especially true for galls that are fusiform in shape but less true for those which are knot-like, because in galls of the latter type the disturbance is general throughout the greater part of the circumference of the stem.

As a usual thing the cells composing the xylem have an upright position, but occasionally there are cells or groups of cells which are turned over at right angles to their normal position. As was stated earlier in this article, the cambium takes a more wavy course than in the normal stem. This is due to the fact that the central portions of the stem bundles grow more rapidly in the radial direction than do the flanks of the bundles, so that the cambium is bowed outward opposite the central portions (fig. 8). The leaf trace bundles, on the other hand, are often inhibited in their growth and the cambium opposite them extends inward farther than in normal stems. Sometimes the cambium is obliterated for a space, or at least there are no cells which have the form of cambium cells. In cases of this kind it is difficult to find a separation between wood and bark.

The normal phloem (fig. 6) has both sieve tubes and companion cells well marked. Where abnormalities are great in the gall all distinction between sieve tubes and companion cells is lost and the cells have increased enormously in size. Figure 7 was drawn from such tissue and is on the same scale as figure 6. The cambium is towards the lower side in figure 7, and shows the rapid increase in the size of the cells after they are formed. The phloem is here composed of undifferentiated parenchyma which has lost all the characteristics of phloem cells. In longitudinal sections through the phloem there are no indications of sieve plates and the individual cells are about isodiametric. In cases in which the bundles have become infected rather late in their growth there is a mass of normal phloem next the bundles of bast fibers, while that next the cambium is similar in structure to that shown in figure 7.

In the normal stem a bundle of bast fibers usually occurs opposite each fibrovascular bundle. In the gall this is not always true, group of parenchyma cells replacing the bast of fibers. Sometimes in such places the bast fibers are imperfectly formed, in that the walls are thinner and less regular in shape and they still contain protoplasm. These cells are often scattered about through the inner bark and are not arranged in well defined bundles as is the case with normal bast fibers. Well formed fibers sometimes occur among the abnormal ones.

The cortical portion of the bark is usually not greatly changed and as a rule there are but few sporanges in it. The cells often become lengthened tangentially and cross walls form in many of them but otherwise there is no great change. Where the bark has been torn open by pressure from within there are sometimes callus-like masses, but whether these are due to the wound stimulus or to the gall stimulus can not be determined. Irregularly shaped cavities sometimes form in the bark and in other parenchyma portions, evidently due to a breaking down of cells. Somewhat similar cavities also occur in uninfected stems.

A diagrammatic drawing of a tangential section of one of the galls is shown in figure 3. This section shows about average conditions; greater tissue disturbances occur in some galls, in others less. By comparing this figure with that of the normal stem (fig. 1) it is seen that the most striking changes brought about in the gall are the broadening of the rays and a reduction in the width of the bands of fibers and other elements between the rays. There is also a vertical shortening of some of the rays, but this is not especially pronounced. The cells which compose the rays are in general more nearly isodiametric than those in the normal rays, many of whose cells are lengthened vertically. The vessels pursue a more irregular course than in the normal stem, and the vessel segments are much shorter. A sharp turning of the fibers from their usual upright course, which is pronounced in the traumatic wood of this species (fig. 12), occurs but seldom. There are, however, occasional bundle ellipses (fig. 9), which in their more complex form consist of several vessel segments so bent as to form a more or less oval or elliptical body enclosing short tracheids. It is probably true that the larger segments are more nearly like tracheids, as distinct membranes often separate one segment from another. Some of the segments in figure 9 show this condition. Bundle ellipses do occur which are much simpler in structure than the one figured. These sometimes consist of as few as three segments which do not enclose still other cells at the center. Irregularly shaped masses of short segmented vessels also occur variously wound and twisted together.

One gall was found which had been severely wounded. A longitudinal slit had been formed in it extending to within a short distance of the pith. On each side of the wound, parallel with its edges, there are strands of fibers, parenchyma, and short segmented vessels. These have been turned over at right angles to their usual position and extend outward in a radial direction. A small cavity at the inner end of the wound has become surrounded by callus tissue, and a similar modification has taken place along the edges of the wound in the outer bark. Tangential sections through this wounded gall show broad ray-like masses of parenchyma with suggestions of fiber inclusions similar in some respects to the condition found in traumatic wood (fig. 13). Resting as these observations do on a single specimen, it is hardly safe to draw any general conclusions from them. It looks, however, as though the wound stimulus were able in a way to overcome the gall stimulus and to imprint its effect on the tissue formed after wounding, or at least to modify to a certain extent the effects of the gall stimulus.

GALLS OF PAPAIPEMA NITELA ON AMBROSIA TRIFIDA

The stems of *Ambrosia trifida* are often parasitized by the common stem borer *Papaipema nitela* Gn., causing the formation of galls. The galls consist of slight swellings which are often fusiform in shape. The general features of such a gall are shown in the diagrammatic drawing, text-figure 1 A, which represents a gall formed by both Protomyces and apaipema. This being the case, no other drawing was necessary except for special features which are shown in figures 2 and 11.

The structural abnormalities caused by this insect are less striking than those caused by Protomyces. There is a greater radial growth of the xylem portions of the bundles than in the normal stem, leading to the production of a more or less compact woody mass of tissue as shown in the lower part of text-figure 1 A. In this radial growth the bundles lose their individuality as such while they remain separate a short distance above and below the gall. Text figure 1 B is an outline drawing of one of the stem bundles taken just below a gall. The rays are broader, as shown in figure 2, and in some instances there is a great reduction in the number of vessels. There is also a considerable production of parenchyma where normally there are only fibers.

An interesting feature occurs in many of the galls which is shown in figure 11. In many instances it was found that the ray cells had proliferated, forming callus-like masses which extend into the larval chamber. These masses consist of thin-walled parenchyma cells which are often very much elongated. Among the large cells there are still other cells which are very much smaller, and some of these are cambiform and appear to have been recently capable of division. A band of these cambium-like cells often extends across the base of the parenchyma mass and probably is capable of renewing the mass. The cells both large and small are poor in protoplasmic and other contents.

Cosens (1, figs. 17 and 21) shows somewhat similar tissue-masses extending into the larval chamber of two other lepidopterous galls, *Stagmatophora ceanothiella* Cosens on stems of *Ceanothus americanus*, and *Eucosma scudderiana* Clements on stems of *Solidago canadensis*. In the case of the Eucosma gall, at least, he regards these growths as nourishing tissue for the larva, for he states (p. 312): "The gall mass in this case is produced from the vascular bundles and the intervening parenchymatous strands. When the larva first enters the stem it first eats out the pith. After the exhaustion of this source of nourishment, its food is supplied by the radial thickening of the bundles into the gall cavity."

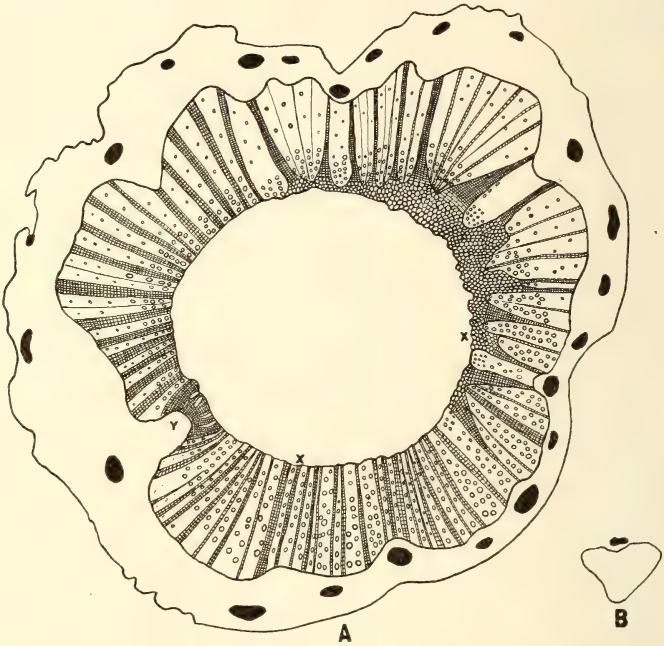
In the gall considered in the present article the cells are very poor in contents which make up the masses, a condition hardly to be expected if they were designed especially as food for the larva. Furthermore, the larva is able to, and does, eat out the pith for a considerable distance beyond where the gall occurs. That they are formed especially for food seems unlikely, as they contain but little substance that could be used for food. The great similarity between these parenchyma masses and callus tissue leads one to suspect that possibly the wound stimulus, resulting from the gnawing of the insect, might have caused them. Indeed, similar instances have already been reported. Küster (3) discusses this phase of the subject at some length. In one instance (p. 279), he states: "Auch in ihren Entwicklungsstadien können die Gallen noch durch Wundreize beeinflusst werden. Im Innern die Potania-Gallen wird das zartwandige Parenchym, das die Larvenkammer auskleidet, und dessen Zellen den Fresswerkzeugen der Gallbewohner zum Opfer fallen, immer wieder durch callusartig Wucherungen regeneriert." There is also a possibility that the callus-like masses result from a stimulus the object of which is to fill the gall cavity with tissue. Küster (p. 314) mentions instances of this kind in which the gall had been abandoned by the insect.

In young galls, where the pith was not entirely eaten away, a ring of cambium-like cells was sometimes found near the border of the cavity. The bundles themselves are sometimes able to proliferate inward, the condition in this respect being similar to that described by Cosens from the gall of *Eucosma scudderiana.* Where conditions of this kind occur there is usually xylem present next the cambium ring, but a short distance inward the character of the cells changes and there is nothing but parenchyma. Sometimes the parenchyma extends to the cambium ring for a considerable space, having entirely replaced the xylem. Bands of cambiform cells occur in these parenchyma masses similar to those which occur in the callus growths already described. They are both eaten by the larva to some extent, but it seems unlikely that they are formed by the plant for this purpose through some stimulus from the larva.

Gall of Protomyces andinus and Papaipema nitela on Ambrosia trifida

In the thicket from which the Protomyces galls described in this article were taken there were also plants of *Ambrosia trifida* which bore galls of *Papaipema nitela.* I was fortunate enough to find several galls in which both fungus and insect were present, and from the structure of these it was evident that both organisms had contributed to their formation. In some of these galls the fungus was confined to a sector of the stem, as was shown by the presence of the sporanges in this part only. From facts already mentioned in this article, we can safely assume that the opposite side of the gall, which contained no sporanges, had not been influenced by the fungus in its development. A diagrammatic drawing of a cross section of one of these galls is shown in text-figure 1. On the lower side of the figure, between the letters X-X, there is a sector, including about one fourth of the stem, which has no sporanges or other indications of the fungus in it. The remainder of the section has the fungus present in it to a greater or less extent.

The structure of the portion on the lower right side is similar to that of galls formed entirely by the insect. There is a greater radial growth of the bundles, as can be seen by comparing the width of the bundles with that of a normal bundle from the same stem (text-figure 1 B). The radial growth of the bundles is often more than twice normal. The inner extremities of the bundles have been eaten away in all but two cases, on the right side just below X where a small part of the pith remains. The bark on this part of the gall is but slightly thickened, being similar in this respect to galls which are formed entirely by the insect. To the left and above the area indicated by the letters, all the tissues of the stem have come under the influence of the fungus to a greater or less extent. The invasion of the fungus has been slow in a peripheral direction, and the inner ends of several bundles, on each side of the area X-X, show the characteristic structure of the xylem of the insect gall. The structure of the outer parts of the bundles, on the other hand, shows abnormalities commonly found in the Protomyces gall, especially the greater production of parenchyma and the reduction in number and size of the vessels. A deep depression occurs on the lower left side, at Y, caused by the suppression of one of the leaf trace



TEXT-FIG. 1. *A*, Diagram of a cross section of a gall formed by *Protomyces andinus* and *Papaipema nitela* (× 27). *B*, outline of a single bundle taken from a section just below the gall (× 27). Description of both drawings in text.

bundles. This area is filled with a mass of parenchyma in which there are a few fibers but no vessels. There is a slight proliferation of the parenchyma into the gall cavity similar to that which has been described from the insect gall. There are no fungus sporanges immediately adjacent to this proliferation, but they do occur close to what was formerly the cambium. Opposite the parts occupied by the fungus the bark is very irregular and thickened, as can be seen by comparing the two sides of the figure.

We have well illustrated in this gall the effects of the two stimuli, fungus and insect, working together. Of the two, the stimulus from the fungus seems to be the stronger. In portions of the stem where the fungus was present very early in the growth of the bundles, all the structural characteristics are present which are commonly found in galls formed by the fungus alone. On the side of the stem where the fungus was not present in the early growth the structure is the same as in the insect gall. As the fungus spreads in a peripheral direction, however, those bundles which have already responded to the stimulus from the insect in their early growth immediately assume the structure of the fungus gall in the tissue formed after invasion. When the two stimuli act together on young tissue it seems to be true that only that from the fungus is capable of causing change in structure. When the fungus invades tissue already under the influence of the stimulus from the insect, the influence of the latter is destroyed or neutralized and the tissue formed subsequently is that of the fungus gall.

It is unfortunate in this case that we have to do with an insect gall former which is incapable of causing violent disturbances in the host plant. It would be interesting to know what would be the result on the tissues of the host plant if two organisms, a fungus and an insect both capable of producing profound tissue modifications, should exert their influence at the same time.

Traumatic Wood of Ambrosia Trifida

It is beyond the scope of this article to enter into a description of the various types of traumatic wood which result from the different kinds of wounds such as has been given by deVries (2) and Mäule (4), because only general features of the traumatic tissue are of interest in this connection. It might be well to mention, however, that the types of wound wood examined are quite similar in a general way to types described by these authors.

Wood resulting from two sorts of wounds was examined. One of the wounded stems was collected along a roadside where a vehicle or some other moving object had torn away one side of the stem which had later started to heal over. The other was of a stem which had been broken over and badly twisted. The first of these stems was examined from cross and tangential sections, the second from tangential sections only. The cross section of the first shows a solid mass of wood in the wounded area, 8 mm. thick at the widest point, which had lost all appearance of having come from a stem normally herbaceous. The vessels are smaller than in the normal stem and are reduced in number. With the exception of occasional broad ray-like masses of parenchyma, the rays are but little broader as a whole than they are in the normal stem. In tangential section the rays are shortened vertically. As a whole there is less ray tissue per unit of area than in the normal stem (fig. 12). There are occasional broad ray-like masses of parenchyma (shown by the heavy lines in the ray, fig. 13), which call to mind very strikingly the similar condition that has been described in the rays of some of the lower angiosperms. The vessel segments are much shortened (compare figs. 1 and 12). The only features worthy of special mention found in the stem which had been twisted are the bundle ellipses, one of which is shown in figure 10. They are very similar to the bundle ellipses in the Protomyces gall (fig. 9).

General Summary

After having considered the structural changes brought about in the Ambrosia stem through the action of the different stimuli treated in this article, it seems desirable to give a brief comparison and summary of the results. One of the more marked features of pathologic plant tissue is the change in general direction of the cells, and in the cases being considered this is more marked in the truamatic wood. An interesting thing in this connection is the fact that the changes brought about by wounding are about the same as those which occur in traumatic tissue of the lower angiosperms. An examination of the literature has revealed practically nothing concerning the structure of wound tissue in the Compositae. A great misplacement of xylem cells takes places, as a result of which they are often 90° or more from their normal position. Gnarl-formations and similar structures result from such misplacements, the bundle ellipses being a marked feature in this respect. With few exceptions the cells in the Protomyces gall occupy more nearly their normal positions and there is but little of the violent misplacement so common in traumatic wood. There are, however, occasional gnarl-like arrangements of cells, best illustrated by the bundle ellipses which are very similar in structure to those of traumatic wood. The insect gall shows no marked misplacement of cells.

Another characteristic feature is the production of parenchyma at the expense of the xylem, a feature which is more marked in the Protomyces gall than in any other. In extreme cases considerable portions of the xylem may be converted into parenchyma. Broad ray-like masses of parenchyma are a constant feature in certain types of traumatic wood. Intermixed with the ray tissue there are often fibers singly or in groups. In the insect gall the production of additional parenchyma tissue is chiefly confined to the proliferation of the ray cells into the gall cavity.

The rays show the greatest alteration in the Protomyces gall, where they are much broader, and the intervening strips of fibers and other elements are narrower than in normal tissue. There is, furthermore, but little vertical shortening of the rays. In traumatic wood, on the other hand, there is a marked vertical shortening of the rays, a feature common to the traumatic wood of most species of angiosperms. The insect gall shows but little alteration in ray structure.

The number and size of the vessels is also greatly influenced by the different stimuli in question. The reduction in number is very marked in both traumatic wood and Protomyces gall, but in the insect gall there is very little change in this respect. There is a noticeable shortening of the vessel-segments in all the types of pathologic tissue under consideration.

As regards the effect of the different stimuli acting together, the insect is able to exert an influence on the growth in all parts of the stem which are in close proximity to the insect. The fungus, on the other hand, is able only to influence growth where the fungus is actually present in the tissue. The insect is therefore able to exert an influence much farther away from the source of the stimulus than is the fungus. When both parasites occupy a portion of the stem at the same time, the insect is able to influence the growth on all sides of the stem from the start, while the stimulus from the fungus is limited owing to the relatively slow spread of the fungus through the tissues. In places where the application of the two stimuli evidently began at about the same time the resulting tissue is the same in structure as that which occurs in the Protomyces gall, the stimulus from the insect being apparently inactive under such conditions. In portions free from the fungus the changes are the same as are usually found in the insect gall. If the fungus extends into such tissue, however, there is an abrupt change in the tissue formed afterwards, this tissue having the same structure as in the Protomyces gall. From these facts we may conclude that the stimulus from the fungus is much the stronger of the two and is able to overcome or neutralize the stimulus from the insect.

Observations on wound and fungus stimuli acting together rest on too few observations to warrant any definite conclusions. It is likely, however, that the wound stimulus is more powerful than the gall stimulus; this at least is suggested by certain modifications in the wounded gall examined. This conclusion is further substantiated by the fact that in general the gall stimulus is not effective for any great distance away from the source of the stimulus, while in the case of the wound stimulus tissue modifications take place at considerable distances from the wound.

The most marked similarities between the structure of the fungus gall and that of traumatic wood are in the presence of bundle ellipses and in the reduction in the number and size of the vessels. Similarities between the insect gall and traumatic wood consist in a greater radial growth of xylem, resulting in a more distinctly woody growth than takes place normally.

This article was prepared in the Department of Botany, University of Wisconsin. The writer wishes to acknowledge the many courtesies shown him by members of the department while the work was in progress. Dr. S. B. Fracker, of the Wisconsin State Department of Agriculture, very kindly identified the organism causing the insect gall.

Florida State College for Women,
Tallahassee, Florida.

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EXPLANATION OF PLATE II

All drawings are from the stem of *Ambrosia trifida* L. Figures 4, 5, 6, 7, 9, 10, and 11, show detailed structure. Because of the low magnification the remainder of the drawings are diagrammatic.

Fig. 1. Tangential section through wood of normal stem. × 28.

Fig. 2. Tangential section through gall of *Papaipema nitela*. × 28.

Fig. 3. Tangential section through gall of *Protomyces andinus*. × 28.

Fig. 4. Longitudinal section through gall of *Protomyces andinus* showing structure of tissue strands. × 133.

Fig. 5. Cross section of tissue strand from gall of *Protomyces andinus*. × 133.

Fig. 6. Cross section of normal phloem. × 133.

Fig. 7. Cross section of phloem of gall of *Protomyces andinus*. × 133.

Fig. 8. Cross section of gall of *Protomyces andinus*. The upper portion alone shows abnormalities. × 6.

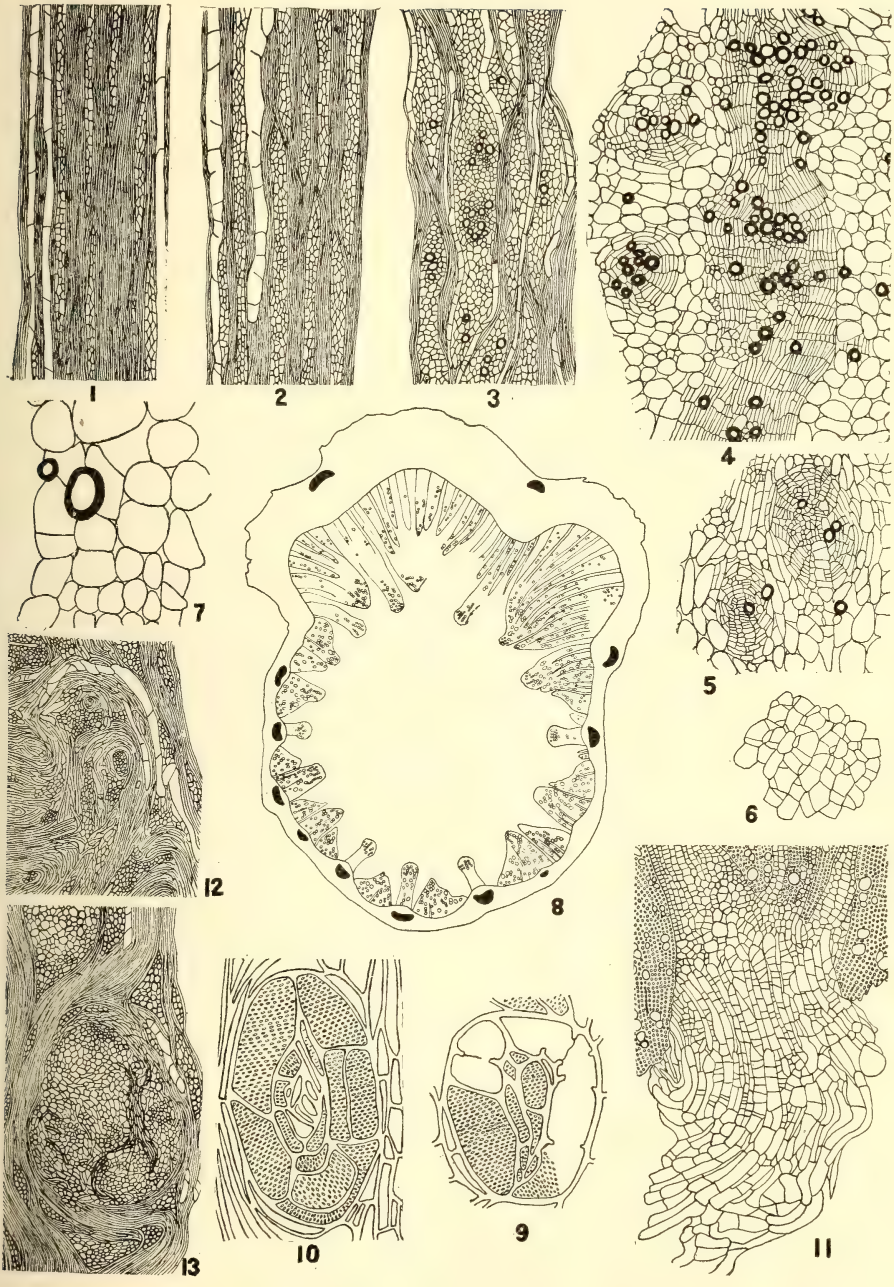
Fig. 9. Bundle ellipse from gall of *Protomyces andinus*. × 133.

Fig. 10. Bundle ellipse from traumatic wood. × 133.

Fig. 11. Proliferation of ray tissue into gall cavity in gall of *Papaipema nitela*. × 133.

Fig. 12. Tangential section through traumatic wood showing course of fibers and vessels. × 28.

Fig. 13. Tangential section through traumatic wood showing fiber inclusions in the broad rays. × 28.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STEWART: PATHOLOGIC CONDITIONS IN AMBROSIA TRIFIDA.

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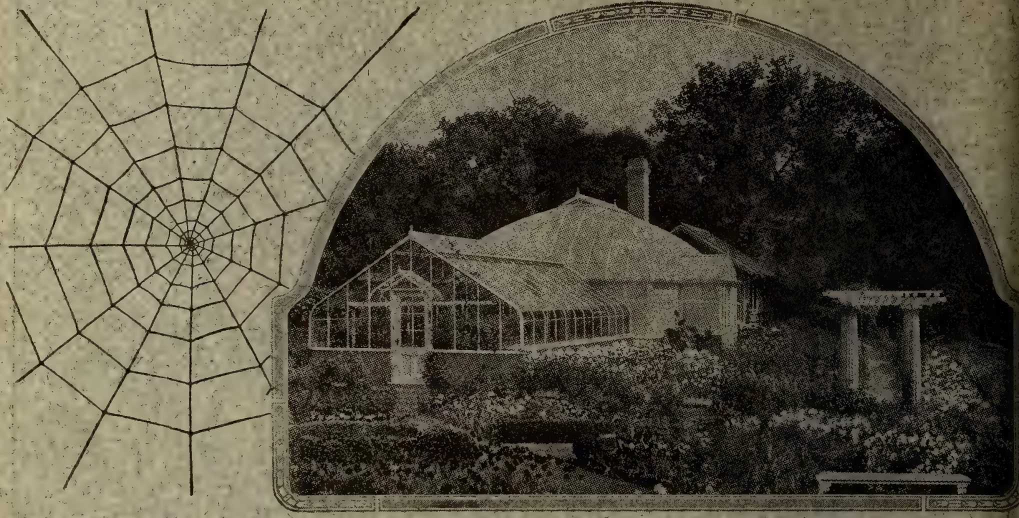

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AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

Smithsonian Institution
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PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT 41 NORTH QUEEN STREET, LANCASTER, PA.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879

AMERICAN JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

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The Journal is published monthly, except during August and September. Subscription price, $5.00 a year. Single copies 60 cents, plus postage. Postage will be charged to all foreign countries, except Mexico, Cuba, Porto Rico, Panama, Canal Zone, Republic of Panama, Hawaii, Philippine Islands, Guam, Samoan Islands, and Shanghai. Postage to Canada 20 cents a volume on annual subscriptions; to all other countries in the Postal Union, 40 cents a volume on annual subscriptions.

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AMERICAN
JOURNAL OF BOTANY

Smithsonian Institution
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VOL. VI FEBRUARY, 1919 NO. 2

CYRTANDREAE HAWAIIENSES, SECTIONS SCHIZOCALYCES HILLEBR. AND CHAETOCALYCES HILLEBR.

JOSEPH F. ROCK

The present paper is the third of a series dealing with the Hawaiian members of the genus Cyrtandra. The two previous papers were published in this **Journal** (**4**: 604–623; **5**: 259–277).

SECTION THREE: SCHIZOCALYCES HILLEBR. FL. HAW. ISL. 325. 1888

Calyx large, split to near the base into more or less equal broadly ovate to linear-lanceolate lobes. Flowers either single or three rarely more in a corymbose cyme (*C. Grayana*) or umbel (*C. umbracculiflora*). Leaves linear, lanceolate to obovate-oblong or broadly ovate. Tomentum fulvous or deep ferruginous.

Hillebrand classed under Section Schizocalyces the following species: *C. macrocalyx*, *C. lysiosepala*, *C. Grayana*, *C. procera*, *C. Lessoniana*, *C. biserrata*, *C. kauaiensis* and *C. Hillebrandi;* of *C. lysiosepala* he enumerates three varieties: *β*, *γ pilosa*, and *δ*; of *C. Grayana* a var. *β latifolia;* of *C. Lessoniana* a variety *β*, *γ angustifolia*, and *δ pachyphylla*. Clarke described a new species as *C. Grayi*, which had to be referred to *C. lysiosepala* where it is retained as a variety *Grayi* in this paper. Hector Léveillé described as new two species *C. Fauriei* and *C. kamoloensis*, which come under the section treated in this paper. The former is identical with Hillebrand's variety *δ*, of *Cyrtandra lysiosepala* (Fl. Haw. Isl. 330. 1888); it is not specifically distinct and is therefore retained with the name *Fauriei* as a variety of *C. lysiosepala;* the second species is identical with *Cyrtandra Grayana* and is consequently reduced to synonymy. Hillebrand's varieties of *C. Lessoniana* have been incorporated with the species because the numerous intermediates do not permit of their being retained as distinct varieties. Hillebrand's var. *β* of *C. lysiosepala* was given the name *latifolia*. The writer has added four new species, *C. halawensis*, *C. kohalae*, *C. Conradtii*, and *C. umbracculiflora*, which are here described for the first time. The first and third are peculiar to the island of Molokai, the second to the oldest portion of the island of Hawaii, and the fourth to the island of Kauai. To the existing varieties he has added also four new ones: of *C. lysiosepala* a variety *haleakalensis* from

[The **Journal** for January (**6**: 1–46) was issued Feb. 1, 1919.]

East Maui; and of *C. Grayana* three varieties, var. *linearifolia* from West Maui, var. *lanaiensis* from the island of Lanai, and var. *nervosa* from Mt. Puukukui, also West Maui. The island of Kauai has furnished this section with one of the most distinct species with no immediate or close relatives or varieties of any sort. Molokai has furnished two species: one, *C. halawensis*, is related to *C. Grayana* but is certainly distinct enough to be classed as a species; *C. Conradtii* belongs in a class with *C. lysiosepala*, but has the appearance of *C. paludosa* or *C. longifolia*, the specific limits here not being as well defined as in the case of the species from Kauai. Hawaii furnishes one species, *C. kohalae*, which must be classed with the present section, but is in habit similar to the Crotonocalyces. It is the only representative of this section on Hawaii. We see from the distribution of the species of this section that *C. Grayana* has taken possession of the central group of islands, predominating on the western end of Maui where the species and most of its varieties occur. The remaining varieties of *C. Grayana* are to be found in the immediate neighborhood of West Maui, as for example diagonally across only a few miles distant, on the western end of Molokai (var. *Fauriei*), and immediately across to the south of West Maui on the island of Lanai, only a few miles distant (var. *lanaiensis*). Its closest relatives are found also on the western end of Molokai in Halawa Valley (*C. halawensis*), and on the more distant summit range of Pelekunu-Waikolu in the central part of Molokai (*C. procera*). Its nearest relatives on Oahu are probably *C. Oliveri* (*C. Hillebrandi* Oliver) and the specifically more remote *C. Lessoniana*. Most of the species of Cyrtandra found on Hawaii belong to the group of *C. platyphylla;* there are, however, two other species: *C. Menziesii*, belonging to Section Chaetocalyces, and one other, belonging to Section Microcalyces. *Cyrtandra lysiosepala* has a distribution similar to that of *C. Grayana*, being restricted with its varieties to the two central islands, Maui and Molokai. Variety *pilosa* from Maui seems to bring *C. lysiosepala* close to *C. waiolani* from Oahu.

CYRTANDRA LESSONIANA Gaud. Voy. Uranie 447, tab. 54. 1826

A shrub 1.75–3 m. high, branches terete to subquadrangular, young branches and inflorescence fulvo-silky-tomentose; leaves opposite, elliptical-oblong to obovate-oblong, acute at both ends, minutely denticulate, thin to thick-chartaceous, glabrate or sparsely pubescent above, silky-tomentose underneath with yellowish-brown hair, especially along the midrib, veins, and nerves, 6–21 cm. long, 2–5 cm. wide, on petioles of 1–3.5 cm.; flowers usually single in the axils of the upper leaves, drooping, rarely two, on a peduncle of 1–2.5 cm.; pedicels 1–2 cm.; bracts oblong to linear-lanceolate, 0.6–1 cm. long; calyx thin, whitish, glabrate or hirtulose, divided nearly to the base into broadly ovate acute lobes, these angular-valvate in the bud, open and quite reflexed with fruit; corolla exceeding the calyx often by one third, sometimes as long as the calyx, 20–25 mm. long, straight-tubular, ampliate above, with short obtuse lobes, the entire corolla covered with long whitish silky hair, glabrous inside or puberulous; ovary ovoid, glabrous, style short, articulate below the deeply lobed, papillate stigma; fruit ovoid, not exceeding the calyx, glabrous.

INS. SANDWICH: Gaudichaud in herb. De Candolle, Paris, Berlin, Delessert, and Gray.

OAHU: Macrae, in herb. Kew, De Candolle, British Museum, Berlin, Vienna; Capt. Beech. Voy. in herb. Kew, Vienna; Nuttall in herb. Kew, British Museum; U. S. Expl. Exped. in Gray Herbarium; Hillebrand no. 331 in herb. Kew, Berlin; Meyen Reise in herb. Berlin; Kaala Mts., Oahu, Mann and Brigham no. 617 in herb. Cornell University and Gray Herbarium; Wawra on Mt. Waiolani, no. 1692 in herb. Vienna and part in herb. College of Hawaii; Heller on Konahuanui, back of Honolulu, at elevations of 1,500 to 2,000 feet, nos. 2896, 2300a, and 2351 in Gray Herbarium and herb. Cornell University; Rock, Punaluu Mts., Koolau range, dense wet forest, flowering Aug. 8, 1908, no. 14, Dec. 7, 1908, nos. 140, 150, 305, Oct. 31, 1914, no. 13072, in herb. College of Hawaii; Rock, Kaukonahua Gulch near Wahiawa, May 15, 1909, no. 3055 in herb. College of Hawaii; Rock, Waikane Mts., windward side, Jan. 23, 1909, no. 1136 in herb. College of Hawaii; Rock, trail to Mt. Konahuanui, Jan. 7, 1909, no.1066 in herb. College of Hawaii; Abbé Faurie, Punaluu Mts. May, 1910 (no. 636 Faurie), no. 13072 in herb. College of Hawaii; A. S. Hitchcock, Schofield Barracks, eastern range, July 11, 1916, no. 14024 in U. S. National Herbarium; A. S. Hitchcock, Kalihi Valley, Honolulu, Aug. 2, 1916, no. 14108 in U. S. National Herbarium.

Cyrtandra Lessoniana is inclined to be rather variable in size and shape of leaf as well as in size of corolla and calyx and length of peduncle and pedicels. There are a few instances in which an intermediate stage between *C. Lessoniana* and *C. Pickeringii* is exhibited in one or two specimens. To these must be referred the writer's no. 330 from the Koolau range, Punaluu mountains; in this plant the leaves are small and ovate, the calycine lobes are large and similar to those of *C. Lessoniana* but not whitish and thin. The plant is a form of *C. Lessoniana*. The specimens from the Koolau, Punaluu Mountains, have narrower, lanceolate leaves, while Hitchcock's no. 14108 from Kalihi has the broadest leaves. Hillebrand's var. β of this species is evidently only a form of it. Hitchcock's no. 14024 from the Waianae range (Schofield Barracks) would be referable to Hillebrand's var. β. His varieties *angustifolia* and *pachyphylla* cannot well be retained as we have numerous intermediates with leaves ranging from linear-lanceolate to obovate-oblong to ovate leaves, with corresponding variation in the calycine lobes. The species is confined to the island of Oahu. Asa Gray's var. β (Proc. Amer. Acad. **5**: 352. 1861) from West Maui does not belong here, but must be classed with forms of *C. lysiosepala*.

CYRTANDRA LYSIOSEPALA (A. Gray) C. B. Clarke. De Cand. Monogr. Phan. 5: 225. 1883–1887

Cyrtandra triflora Gaud. var. γ *lysiosepala* A. Gray, Proc. Amer. Acad. 5: 351. 1861.

A shrub 2.5 to 3 m. high; branches obscurely quadrangular, the young parts brownish-pubescent with short hairs; leaves opposite, the upper ones rarely ternate, membranous, elliptical, acuminate at both ends, with serrate margins, pilose above, villose-pubescent below, with yellowish hairs, especially on midrib and veins, 8–14 cm. long, 5–6 cm. wide, on petioles of 15–40 mm.; inflorescence a three-flowered cyme, the peduncle 3–4 cm.; bracts lanceolate, 1 cm.; pedicels 10–15 mm.; calyx white, parted to the base into spreading, broad-lanceolate to spathulate lobes, 2 cm. long, 3–5 mm. broad; corolla partly villose, the tube erect, the lobes large and spreading; ovary glabrous, style short; stigma broadly lobed; fruit glabrous, shorter than the calyx, ovoid, sessile.

HAWAII: In deep forest, U. S. Explor. Exped.

MAUI: West Maui, Kaanapali, Hillebrand (three sheets) in herb. Berlin; East Maui, southern slopes of Haleakala, Hillebrand no. 326 in herb. Kew and Berlin; Honomanu Valley, northeastern slope of Mt. Haleakala, flowering, May 1911, Rock, in herb. College of Hawaii.

The specimen in Hillebrand's collection in the Berlin Herbarium, from East Maui, bears Clarke's signature and is either the type or co-type of this species; the specimen in Berlin is without number, while the specimen in the Kew Herbarium bears the number 326. The writer's specimen from Honomanu is identical with the material determined by Clarke, and is the typical *C. lysiosepala*.

CYRTANDRA LYSIOSEPALA FAURIEI (Lév.) Rock

Cyrtandra Fauriei Lév. in Fedde Repert. Spec. Nov. 10: 123. 1912.
Cyrtandra lysiosepala var. δ Hillebr. Fl. Haw. Isl. 330. 1888.
Cyrtandra lysiosepala var. *molokaiana* Rock ms. in herb. Vienna and Berlin.

A shrub with subquadrangular branches; leaves elliptical, acuminate at both ends, thin, coarsely and distantly dentate, 6–12 cm. long, 2–5 cm. wide, hirtellous, dark green above, fawn-colored-pubescent below, on petioles of 2–4 cm.; inflorescence a 3-flowered cyme; peduncle 2.5–6 cm.; bracts ovate, acute; pedicels about 2 cm., the middle one 2.5 cm.; calyx white, the lobes lanceolate, acute, 3-nerved; corolla hirtellous to glabrate; ovary glabrous.

MOLOKAI: Mapulehu, Halawa, and Kalae, Hillebrand in herb. Berlin, sine num.; Mapulehu Valley, flowering March 1910, Rock no. 10338, in herb. College of Hawaii; Pukoo (Mapulehu), fruiting May 1910, Faurie no. 632, in herb. College of Hawaii (no. 13076).

Plants of this variety were distributed to the herbaria of Vienna and Berlin during the writer's stay in Europe, as *Cyrtandra lysiosepala* var. *molokaiana*. It was, however, described by Léveillé as a distinct species

(*C. Fauriei*). The plant is a mere variety of *C. lysiosepala* and was as such recognized by Hillebrand, who enumerates it as var. δ. It differs from the species mainly in the ternate leaves, and lanceolate acute sepals instead of spathulate ones; otherwise it is the same.

CYRTANDRA LYSIOSEPALA LATIFOLIA Rock

Cyrtandra lysiosepala var. β Hillebr. Fl. Haw. Isl. 330. 1888.

A small tree or shrub, the branches quadrangular, glabrous, but hirtellous at the apices; leaves opposite, broadly ovate, acuminate at both ends, subglabrous above, or with scattered hairs, fawn-colored and pubescent below, dentate to serrulate, 10–15 cm. long, 5–7.5 cm. wide, on petioles of 3–5 cm.; inflorescence a 3-flowered cyme, the peduncle 2.5–3.5 cm., bracts linear-lanceolate, acuminate, pedicels 1.5–3 cm.; calyx lobes subulate (*teste* Hillebrand) but often linear-lanceolate, acuminate, broadest at the middle, 1.5–2 cm. long, 2.5–4 mm. wide; corolla the length of the calyx, hirtellous; ovary ovoid, glabrous, the glabrous style articulate near the ovary; fruit ovoid, glabrous.

MAUI: East Maui, Hamakua, Hillebrand in herb. Berlin; West Maui, Hillebrand, not in herb. Berlin. Lahaina, in a ravine, elevation 1,000 to 2,000 feet, flowering and fruiting Sept. 29, 1916, A. S. Hitchcock no. 14868, in U. S. National Herbarium.

MOLOKAI: Pukoo, tree in rain forest, flowering Oct. 8, 1916, A. S. Hitchcock no. 15031, in U. S. National Herbarium.

Var. *latifolia* comes very close to var. *Fauriei* and could perhaps be classed with the latter. It differs from it in the opposite, broadly ovate leaves. Hillebrand states that the calycine lobes are subulate, which is not the case with specimens from Lahaina, West Maui. West Maui (Kaanapali and Lahaina) is diagonally across from Mapulehu, Molokai, and separated only by a not very wide channel. Birds can easily fly back and forth between these two nearest points carrying the small seeds in their crops.

CYRTANDRA LYSIOSEPALA PILOSA Hillebr. Fl. Haw. Isl. 330. 1888

A small shrub; branches hirsute at their apices; leaves broadly ovate, thin-membranous, acute at both ends, dentate to serrate but entire towards the base, 10–12 cm. long, 4–6 cm. broad, hirsute on both faces; petioles 3–4.5 cm.; flowers single on slender peduncles, hirsute, as are the bracts, pedicel, and calyx; calycine lobes about the length of the corolla, subulate; corolla villous in the upper part, but glabrous below, ovary elongate, glabrous; style glabrous; stigmatic lobes short.

MAUI: West Maui, Kaanapali, Hillebrand, Aug. 1870, in herb. Berlin; East Maui, Hamakua and Ulupalakua, Hillebrand, coll. Lydgate in herb. Berlin; West Maui, Honokawai gulch along stream, shaded by big rocks, elevation 4000 feet, flowering Aug. 25, 1910, Rock and Hammond no. 8157, in herb. College of Hawaii; East Maui, east of Olinda in wet forest along pipe line, elevation 4,000 feet, flowering Oct. 1, 1916, A. S. Hitchcock no. 14913, in U. S. National Herbarium.

HAWAII: Hilo, Hillebrand, not in herb. Berlin.

A distinct variety and almost of specific value. Comes, however, close to the species save in the subulate calycine lobes. The whole plant is very delicate and membranous. It grows in dense shade along streams on West Maui in company with *Gunnera petaloidea*, Kadua, *Schiedea diffusa*, etc. The specimen collected by A. S. Hitchcock near Olinda has small ovate leaves but agrees otherwise with the type.

CYRTANDRA LYSIOSEPALA **haleakalensis** Rock n. var.

A shrub with terete branches; leaves opposite, thin chartaceous, elliptical-oblong, acute at both ends, serrulate, 10–14 cm. long, 3–5.5 cm. wide, dark green above, hispidulous, glabrate to puberulous underneath, greenish, the prominent midrib and secondary nerves brownish and pubescent, on petioles of 3–5 cm.; flowers single, or two on a peduncle of about 1 cm.; bracts linear, green, 7 mm. long, 1.5 mm. wide; pedicels filiform, 1–1.5 cm.; calyx greenish-brown tomentose, divided to the base into narrow lineate, almost subulate lobes, the latter 12–20 mm. long, 1.5 mm. broad, acute; corolla glabrous or slightly hairy, exceeding the calyx, funnel-shaped, the lobes large and spreading; ovary glabrous, elliptical; style articulate near the base.

MAUI: Ukulele trail to Honomanu, slopes of Mt. Haleakala, flowering Sept., 1910, Rock no. 8564, in herb. College of Hawaii.

A very graceful and delicate variety of *Cyrtandra lysiosepala*, from which it is mainly distinguished by the thin subglabrate leaves, slender peduncle and pedicels, the narrow linear calycine lobes, and single flowers (rarely two).

CYRTANDRA LYSIOSEPALA GRAYI Rock

Cyrtandra Grayi C. B. Clarke in DC. Monogr. Phan. **5**: 218. 1883–1887.
Cyrtandra triflora forma *typica* Wawra (not Gaud.) Flora **30**: 563. 1872.
Cyrtandra lysiosepala Hillebr. (not C. B. Clarke) Fl. Haw. Isl. 330. 1888.

Leaves elliptical-oblong, acute at the apex, acuminate at the base, 15–18 cm. long, 5 cm. wide, irregularly dentate to serrate, thin-membranous, hirtellous above, fawn-colored-pubescent below, on petioles of 6 cm.; inflorescence a 3–6-flowered cyme; peduncles about 2 cm., the bracts small, 8 mm., linear-lanceolate, the pedicels of varying length, 2.5 cm. with fruit, the lobes linear-lanceolate, acute, white, hirtellous, about 1 cm.; fruit ellipsoidal, acute, glabrous.

MAUI: "*Um Waihee*," flower buds and fruiting, Wawra no. 1820a in herb. Vienna and part in herb. College of Hawaii.

According to Clarke the following specimens have been referred to his *C. Grayi* by himself: Hillebrand no. 330, in herb. Kew; Seemann, from Oahu, no. 2277, in herb. Kew; Barcley, in herb. British Museum; and Wawra no. 1820, in herb. Vienna.

From the latter specimen (Wawra no. 1820a) the College of Hawaii possesses parts, as leaves and inflorescence. The writer has also examined Wawra's plant which bears Clarke's determination; it is obvious that the specimen belongs to *C. lysiosepala* and cannot be retained as a distinct species, but must be considered a variety. It differs from *C. lysiosepala* in the longer leaves and petioles, as well as in the more than 3-flowered cyme (Clarke states 3–12-flowered); the sepals are also smaller and acute instead of spathulate.

Cyrtandra Conradtii Rock n. sp.

A shrub, the branches terete to subquadrangular near the apex, hirtellous; leaves ternate, lanceolate-oblong, 20–26 cm. long, 6.5–8 cm. wide, thin-chartaceous, acute at the apex, gradually merging into a petiole of 2–3.5 cm., hispidulous above, glabrate to minutely pubescent below, but distinctly pubescent along the prominent midrib and veins, the margin sharply serrate; calyx white, divided to near the base into linear, subacute to obtuse lobes, 12–14 mm. long, 3–4 mm. wide, hirtellous, distinctly nerved; corolla glabrous to puberulous, slightly exceeding the calyx, the tube straight, the lobes oblong; ovary elongate, acute, glabrous as is the style; stigmatic lobes ovate, glabrous.

MOLOKAI: Mapulehu Valley, 300–400 feet elevation, flowering March 1910, Rock no. 10340 (type), in herb. College of Hawaii.

This species is named for Mr. C. C. Conradt of Mapulehu, Molokai, to whom the writer is indebted for many courtesies and without whose hospitality the exploration of this wonderful valley could not have been accomplished so successfully. *Cyrtandra Conradtii* is related to *C. lysiosepala* but has the habit and appearance of *C. longifolia* var. *degenerans*, or of *C. paludosa*. The calyx is however that of *C. lysiosepala*. The leaves are rather large, ternate, and sharply serrate, and the calycine lobes large and obtuse. Mapulehu Valley and the mountains immediately above it are very rich in Cyrtandreae.

CYRTANDRA BISERRATA Hillebr. Fl. Haw. Isl. 329. 1888

A shrub 1.5–2 m. high, the branches and inflorescence hirsute with spreading rust-colored hairs; leaves quaternate, green on both faces, papilloso-hispid on both faces, elliptical-oblong, 10–12.5 cm. long, 3.75–5 cm. wide, on petioles of 2.5–5 cm., cuspidate, acuminate at the base, deeply and unevenly serrate, almost laciniate; peduncles 8–12 mm. long, 2-flowered, the pedicels 12–16 mm., the lanceolate bracts 6 mm.; calyx thin, hairy, 8–10 mm. high, parted deeply into five lanceolate, acute lobes; corolla 14–16 mm. long, villous, exserted, the slender tube slightly curved and ampliate at the throat, the large spreading lobes bilabiate; ovary glabrous, the style 6 mm. long and broadly lobed; berry ovoid, 18 mm.

MOLOKAI: Hillebrand in herb. Berlin and Gray Herbarium, without locality, date or number, part of type in herb. College of Hawaii; Pukoo, Mapulehu, flowering Oct. 8, 1916, A. S. Hitchcock no. 15011, in U. S. National Herbarium, part in College of Hawaii Herbarium.

A very distinct species re-collected only by Prof. A. S. Hitchcock. The leaves are not always biserrate but deeply and irregularly serrate, also sometimes ternate instead of quaternate.

CYRTANDRA KAUAIENSIS Wawra Flora 30: 566. 1872

A branching shrub, the branches slender, foliose at the apex; leaves membranous, oblong-elliptical, acuminate, subentire, hirsute-pubescent above, the nerves and petiole tomentose with an appressed ferruginous-velvety tomentum, glabrate between the nerves, 7.5–10 cm. long, more than 2.5 cm. broad; petiole about 2.5 cm. long; peduncle 1-flowered, of the length of the petiole, thick, with two caducous filiform bracts above the base; calyx segments foliaceous, green, patent, about 2.5 cm. long, ovate-lanceolate or lanceolate, contracting at the base into short tomentose stipes; corolla exceeding the calyx, the tube partly hirsute, glabrous inside, the lobes ovate, acute, one-third as long as the tube; ovary glabrous; fruit oblong, 1.75 cm., apiculate.

KAUAI: "*Waelder von Halemanu*," Wawra, type, no. 2058, in herb. Vienna, part of type in herb. College of Hawaii; gulch above Waimea, between the forks of the Waimea river, elevation 2,000 feet, Sept. 30, 1895, A. A. Heller no. 2829, in Gray Herbarium.

This species has been re-collected only once since the time of Wawra, and Hillebrand states "not seen by me." Wawra says "ovary glabrous"; in his specimen the ovary is not glabrous but hirtellous and hirsute towards the apex especially at the base of the style; the calycine lobes are thin-membranous, with undulate margins, prominent midrib, and anastomosing veins, hirtellous on both sides but especially along the nerves.

CYRTANDRA PROCERA Hillebr. Fl. Haw. Isl. 329. 1888

Cyrtandra arborescens Hillebr. in mss.

Arborescent, 4–6 m. high, the fleshy branches hirsute with dark ferruginous hairs; leaves in whorls of six, linear-oblong or lanceolate, 12.5–15 cm. long, 2–5 cm. wide, on petioles of 6.5 mm., acute, sharply and finely serrulate, truncate at the narrow base, coriaceous, dark green and glabrous above, thickly tomentose underneath; flowers one, two, or three in each axil on a short peduncle of 2–8 mm., the pedicels 16–24 mm.; bracts 6 mm., linear-lanceolate; calyx thickly tomentose or hirsute, 12–18 mm., split to near the base into five to six linear-lanceolate lobes; corolla 16–18 mm., villous, slightly curved, the large spreading lobes somewhat acute, about 5 mm. long; ovary glabrous.

MOLOKAI: Pali of Pelekunu at a height of 3,000 feet, Hillebrand in herb. Berlin and Gray Herbarium, and part of type in herb. College of Hawaii; near Pelekunu above Kamolo, flowering March 1910, Rock no. 10341, in herb. College of Hawaii; at the head of Waikolu Valley, fruiting May 27, 1918, Rock no. 14077, in herb. College of Hawaii.

Cyrtandra procera is recorded by Hillebrand as being arborescent.

The writer collected this species at the summit ridge of Molokai overlooking the cliffs of Pelekunu Valley. It grows on moss-covered tree trunks and on tree ferns together with *Viola robusta*, and occasionally grows quite tall and has straight ascending branches. It is related to *C. Grayana* but differs from it in the single flowers (rarely three), and the peculiar linear truncate leaves which are on petioles of only a few millimeters. With its dark green leaves which are usually convex with the margins rolled downward, and its straight ascending branches, it presents a rather peculiar aspect in the somber rain forest of the heights of Pelekunu. The writer found it also growing at the head of Waikolu Valley at an elevation of 3000 feet; there it was a sparingly branching shrub with horizontal and slightly ascending branches. The species is peculiar to the heights of Molokai.

The specimen in the Gray Herbarium, ex coll. Hillebrand, is labeled *Cyrtandra arborescens* sp. n.; the specimen is 3-flowered immediately below the uppermost leaf whorl.

CYRTANDRA GRAYANA Hillebr. Fl. Haw. Isl. 330. 1888

Cyrtandra kamoloensis Lév. in Repert. Sp. Nov. Fedde **10**: 123. 1912.

A shrub 2.5–3.5 m. high; leaves in whorls of four to six, narrow, spathulate, 25–30 cm. long, including the long petiole merging into them, 3.5 cm. wide near the apex, shortly acuminate, almost entire, thick-chartaceous, papillose above, thickly tomentose beneath with appressed yellow hairs; flowers 8–12 in an irregular corymbose cyme, the common peduncle 12–24 mm.; pedicels 18 mm., the broadly lanceolate bracts 12–14 mm., calyx parted to near the base into five oblong, obtuse lobes, the whole calyx thickly covered with a brown-fulvous tomentum; corolla hairy, with spreading lobes; ovary glabrous; berry broadly ovate, acute, little longer than the calyx.

MAUI: Mauna Eeke, 5,000 feet, Hillebrand in herb. Berlin and Gray Herbarium, part of type in herb. College of Hawaii, without date or number; Puukukui, upper forest, flowering, A. S. Hitchcock no. 14748, in U. S. National Herbarium.

MOLOKAI: Kamolo, June, 1910, Faurie no. 646, in herb. Léveillé.

The writer has not collected this species* proper but has collected several varieties of it on West Maui and Molokai. Prof. A. S. Hitchcock of Washington re-collected the typical species on Mt. Puukukui, the highest peak on West Maui. Hillebrand's specimens came from Mauna Eeke, the second highest peak of West Maui. These two mountains are not far apart, but the dense jungle makes it exceedingly difficult to pass from one to the other. Clarke's *C. Grayi* is not identical with *C. Grayana* but is intermediate between the latter and *C. lysiosepala*. It differs from the former in the thinner opposite leaves and membranous acute sepals.

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* Collected since by the writer on Mt. Eeke, September, 1918.

CYRTANDRA GRAYANA **linearifolia** Rock n. var.

A tall shrub of palm-like habit; stems stout, covered with large circular leaf-scars; leaves in whorls of six, linear-lanceolate, 16–20 cm. long including the 2 cm. long petiole gradually merging into them, 1.5 cm. wide, thick, densely brown-tomentose underneath, dark green above, acuminate at both ends; inflorescence on the stem near the ground and on exposed rootlets; flowers arranged in a compound cyme, about 7 mm., bracts ovate-elliptical, acute, these and the whole inflorescence densely brown tomentose; pedicels of very variable length; calyx divided to near the base into linear, acute lobes; corolla tube narrow, ampliate at the throat, the lobes broad and spreading, densely hirsute underneath; ovary linear-oblong, the style short, glabrous; stigmatic lobes broad and thick.

MAUI: West Maui, Honokawai gulch in dense forest, flowering Aug. 1910, Rock and Hammond no. 8201 (type), in herb. College of Hawaii.

The plant here described is almost worthy of specific rank and would perhaps be classed as such by less conservative systematists. The leaves are exceedingly narrow, and the remarkable part is that the flowers are borne at the base of the stem and on exposed rootlets instead of being axillary. No writer on Hawaiian plants had heretofore brought out the facts regarding the location of the inflorescence in some of our Cyrtandreae. There are a number of species in these islands that bear the flowers on exposed roots and along the stem, especially near the ground. Often the writer passed a Cyrtandra which at first glance seemed to be without flower or fruit, which usually occur in the axils of the upper leaves; but on examination they were found to be concealed by foliage and ferns near the ground, often absolutely hidden from sight.

CYRTANDRA GRAYANA LATIFOLIA Hillebr. Fl. Haw. Isl. 331. 1888

A shrub 3 m. high; leaves quaternate, obovate-oblong, acute to acuminate at both ends, 25–30 cm. long including the petiole, up to 10 cm. wide (*teste* Hillebr.); petioles of variable length up to 6.5 cm., thick-chartaceous, hirtellous above, densely brown-tomentose beneath, subentire to denticulate; inflorescence a cyme, the flowers less numerous; calyx-lobes ovate to linear, obtuse, tomentose outside, glabrous inside, 3-nerved; corolla hairy; ovary glabrous; style long, the lobes thin and of the width of the style.

MAUI: West and East Maui, Hillebrand in herb. Berlin, and part of type in herb. College of Hawaii.

MOLOKAI: Mapulehu Mts. and Valley in dense forest along Wailau Trail, 3,000 feet elevation, flowering March 1910, Rock no. 10335; same locality, May 6, 1915, Rock no. 12575, in herb. College of Hawaii.

LANAI: At the head of Mahana Valley, flowering Aug. 2, 1910, Rock no. 8128, in herb. College of Hawaii.

Var. *latifolia* differs from the species in the larger and broader leaves, few (2–5)-flowered cyme, and quaternate leaves. It has not been recorded previously from Molokai. The plants from Molokai have a tendency to bear the inflorescence along the lower part of the stem as well as in the upper leaf-axils. The leaves are only 7 cm. in diameter for the most part, but are as much as 10 cm. in width according to Hillebrand. The Lanai specimens must be referred to var. *latifolia* Hillebr., but they differ slightly from the Maui and Molokai specimens, mainly in the long (4 cm.) peduncle, 2-flowered inflorescence, large bracts, calycine lobes, and corolla. It may be termed a form of var. *latifolia* under the name forma **grandis** Rock n. f.

Cyrtandra Grayana **lanaiensis** Rock n. var.

A much branching shrub, the branches tortuous; leaves quaternate, elliptical, 5.5–10 cm. long, 10–24 mm. wide, acute-acuminate at both ends, the margin distantly denticulate to subentire, thick-chartaceous, hirtellous above, with a dense silky-brown tomentum beneath, on petioles of 1.5–2 cm.; flowers single or three on a common peduncle of 10–25 mm., in the axils of the upper leaves, the bracts linear, acute; calyx lobes lanceolate, acute to obtuse, brown-tomentose; corolla hirsute; ovary glabrous; fruit elliptical, glabrous.

Lanai: Summit ridge of Lanai, Haalelepakai, flowering and fruiting July 25, 1910, Rock no. 8036, in herb. College of Hawaii; on largest mountain, flowering Sept. 21, 1916, A. S. Hitchcock no. 14662, in U. S. National Herbarium.

This variety is a very handsome shrub and differs from the species and other varieties in the small elliptical leaves, short petiole, and short inflorescence, as well as in the smaller flowers.

Cyrtandra Grayana **nervosa** Rock n. var.

A shrub, the branches terete, sulcate when dry; leaves quaternate, elliptical-ovate to obovate-oblong, acuminate at both ends, denticulate excepting the base, thick-chartaceous, hirtellous above, glabrous below with the exception of the very prominently projecting midrib and lateral nerves; inflorescence a short, 3–5-flowered cyme; peduncle 1–1.5 cm.; corolla hairy or glabrate, with large lobes, the inflorescence otherwise the same as in var. *latifolia:* fruits elliptical-oblong, glabrous.

Maui: West Maui, Puukukui, near the summit in dense shaded ravines and gulches, the branches covered with moss, flowering and fruiting Aug. 22, 1910, Rock no. 8172 (type), in herb. College of Hawaii.

This variety is at once distinguished from the others of the species by its leaves, which are glabrous underneath and expose the reticulated network; the midrib and secondary veins are strongly raised and silky brown-pubescent. In the other varieties, as var. *latifolia* and var. *linearifolia*, the whole under surface of the leaf is densely matted with a thick, brown, silky tomentum, which does not show the reticulate network. The plant occurs near the summit of west Maui, elevation about 5,000 feet, in the mossy forest; but the inflorescence is axillary instead of near the base of the stem.

Cyrtandra Oliveri Rock n. name.

Cyrtandra Hillebrandi Oliver in Hillebr. Fl. Haw. Isl. 331. 1888. Not *C. Hillebrandii* C. B. Clarke.

Leaves opposite, coriaceous with stout ribs and veins, elliptical or ovate-oblong, 7.5–12 cm. long, 3–5 cm. wide, on petioles of 2.5 cm., acute, denticulate, pubescent above, faintly ferruginous or glabrate beneath; flowers 3–5, cymosely umbellate on a peduncle of 12–24 mm., the pedicels as long or longer, the bracts ovate-lanceolate, 10–14 mm.; calyx glabrate, thin, 12–18 mm., cleft beyond the middle (deeper on one side), into broad-lanceolate, long-acuminate lobes; corolla little exserted, 18–22 mm. long, pubescent, somewhat curved, with ampliate throat and spreading lobes; ovary glabrous; berry ovoid, elongate, 18 mm. long, enclosed in the calyx.

OAHU: From Nuuanu to Palolo, Hillebrand, in herb. Berlin, and part of type (Nuuanu Valley specimen) in herb. College of Hawaii; Kalihi Valley, flower buds, A. S. Hitchcock no. 14105, in U. S. National Herbarium and part in herb. College of Hawaii.

Cyrtandra Oliveri must be classed with Section Schizocalyces, owing to the thin, deeply lobed calyx. The specific name *Hillebrandi* must be changed owing to Clarke's *C. Hillebrandii* which antedates that of Oliver in Hillebrand's Flora. That *C. Oliveri*, *C. Pickeringii*, *C. honolulensis*, and *C. Hillebrandii* are closely related there is no doubt. The latter has already been classed as a synonym of *C. Pickeringii*. The writer has a large amount of material at hand but the plants are so variable as to thickness, shape, pilosity of leaf, etc., that it is next to impossible to determine them properly. The species mentioned above are in the same state of evolution as are *C. platyphylla* and its numerous varieties on Hawaii. Certain species of Cyrtandra are exceedingly local while others range over the mountains and valleys in various forms, the extremes of which one would be tempted to describe as new species were not the intermediates available. Unfortunately, the writer has not seen the type of Clarke's *C. Hillebrandii*, which is in the Kew Herbarium (Hillebrand no. 329). Drake Del Castillo cites it as a synonym of *C. Pickeringii*. Whether he has compared it with other specimens or has determined the synonymy on the strength of the description is doubtful, though the latter is more probable because his synonymy of other species, for example that of *C. latebrosa*—*C. hawaiiensis*—is quite faulty. The description of *C. Hillebrandii* does, however, differ very little from that of *C. Pickeringii* as understood by Hillebrand. The specimen referred by Hillebrand to *C. Pickeringii* A. Gray has nothing to do with that species, the type of which the writer has only recently been able to examine, but seems to be *C. Hillebrandii* C. B. Clarke. The true *Cyrtandra Pickeringii* A. Gray has apparently not been re-collected; at least the writer did not find it in any of the collections examined by him either in Europe or America. The type of *C. Pickeringii* A. Gray consists of a single leaf and an undeveloped inflorescence; the latter is hirsute with reddish hairs which stand at right angles to the pedicels. The leaf, which is oblong-lanceolate and acuminate at both ends, has the margin densely covered with reddish hairs as are the midrib and veins; the petiole is not tomentose but almost hirsute. The plants referred by Wawra to *C. honolulensis* seem certainly distinct from *C. Pickeringii* A. Gray and must be retained as a good species instead of as a variety of *C. Pickeringii*. The status of all these species is then as follows:

Cyrtandra Pickeringii A. Gray, type in Gray Herbarium, fragmentary.

Cyrtandra Pickeringii Hillebrand (not A. Gray) apparently corresponds to *C. Hillebrandii* C. B. Clarke, no. 329 (type) in Kew Herbarium.

Cyrtandra Oliveri Rock, identical with *C. Hillebrandii* Oliver, the latter being a synonym.

Cyrtandra honolulensis Wawra is a good species, and the combination of *C. Pickeringii honolulensis* Rock must be considered a synonym.

It is best merely to give these notes, which may be checked up by future workers or by the one so fortunate as to have the opportunity to examine Clarke's type of *C. Hillebrandii* in the Kew Herbarium.

Oliver's *C. Hillebrandii* (= *C. Oliveri* Rock) has the calycine lobes divided to the base, while Clarke's *C. Hillebrandii* has the lobes divided to the middle only; as the latter is the only one in Hillebrand's collection with such calycine lobes, it must be taken for granted that it is *C. Hillebrandii* C. B. Clarke. Hillebrand himself identified the particular specimen as *C. Pickeringii* A. Gray; however, since that identification is erroneous, as can be seen on comparison with the type of *C. Pickeringii*, there remain only two suggestions: that it is either an undescribed species, or it is identical with *C. Hillebrandii* C. B. Clarke. The latter is more probable, and this belief is strengthened by the fact that Drake Del Castillo refers to *C. Hillebrandii* C. B. Clarke as a synonym of *C. Pickeringii* A. Gray.

Cyrtandra kohalae Rock n. sp.

A small shrub, the stems and branches quadrangular, hirsute in the upper portion with dark ferruginous hairs; leaves oblong to obovate-oblong, acuminate at the apex, acute and decurrent at the base, the margins irregularly serrate to denticulate, scatteringly pubescent with whitish, 3–5-celled hairlets which disappear with age, velvety underneath with dark brown tomentum, especially along the midrib and veins, 15–20 cm. long, 5–9 cm. wide, on petioles of 3.5–5 cm.; inflorescence in the upper axils of the leaves; peduncle 1.5–2.5 cm. long, bearing either one single flower and without a pedicel or bearing six flowers on pedicels of variable length, brownish-hirsute as are the pedicels; bracts linear-lanceolate, about 2.5 cm. in length; pedicels 5–30 mm.; calyx tube exceedingly short, barely 4 mm.; the lobes filiform to subulate or linear, and long-acuminate, about 2 cm. in length, 1–2 mm. broad; corolla small, about 12 mm. long, tubular and slightly curved, glabrous to hirtulose outside as are the reflected lobes; ovary glabrous as is the articulate style; fruit subglobose to ovate, not exceeding the calycine lobes.

HAWAII: Woods of Kohala, rain forests at an elevation of 4,000 feet; flowering and fruiting June 10–16, 1910, Rock no. 8361 (type), in herb. College of Hawaii (Plate III).

This new species is very near *C. Menziesii* Gray, but differs from it in the coriaceous, velvety-tomentose leaves, the robust habit, and much larger calycine lobes.

Cyrtandra kohalae is apparently related to *C. platyphylla*, from which it differs, however, in the oblong, long-acuminate leaves, but especially in the subulate-linear calycine lobes and small flowers. It is at a glance distinguishable from *C. platyphylla* by the spreading, 2-cm.-long calycine lobes; the tube of the calyx is exceedingly short. This species seems to link together the sections Crotonocalyces and Schizocalyces. The appearance of leaf as well as habit of plant would place this species in the former section were it not for the distinct calyx.

Cyrtandra halawensis Rock n. sp.

A tall shrub, 3 m. or more high; leaves opposite, elliptical to ovate-oblong, acute at both ends, 13–30 cm. long, 7–14 cm. broad, thin-membranous to chartaceous, hispidulous above, silky-brown tomentose beneath, the margins serrate-dentate excepting the slightly uneven-sided base, this merging into a petiole of 2.5–11 cm.; inflorescence axillary, a 3-flowered cyme, yellowish-brown tomentose throughout, the common peduncle 1.5–2.5 cm. long; bracts linear-lanceolate, acute, 5–12 mm.; pedicels of variable length in the same cyme, 4–20 mm. long; calyx lobes linear, 2 cm. long, 2–3 mm. wide, long-acuminate; corolla as long as the calycine lobes or less, of even width, curved and hairy in the upper third; ovary conical-oblong, glabrous, the style articulate below the middle, stigmatic lobes oblong; fruit ovate-oblong, acute, 15 by 10 mm., glabrous.

MOLOKAI: Forests of Halawa Valley on the plateau above the falls, along stream bed and the outskirts of forest, flowering and fruiting April 1910, Rock no. 7010 (type), in herb. College of Hawaii (Plate IV).

Cyrtandra halawensis, while not so distinct a species as *C. umbracculiflora*, is sufficiently distinct to be classed as a species. It is true that it comes close to *C. Grayana* var. *latifolia* Hillebr., but differs from it in the very large, long, linear calycine lobes; the leaf margins are serrate-dentate, while those of *C. Grayana* are subentire.

CYRTANDRA MACROCALYX Hillebr. Fl. Haw. Isl. 329. 1888

Cyrtandra macrostegia Hillebr. mss. (in Gray Herbarium).

Arborescent, 4–5 m. high, the branches moderately hirsute; leaves in whorls of four, elliptical-oblong, acuminate at both ends, serrulate, 10–12.5 cm. long, 3.75–4.5 cm. wide, on petioles of about 12–18 mm., thick-chartaceous, with prominent veins, sparingly hairy above, with a faint ferruginous tomentum beneath; peduncle 8–16 mm., bearing one or two flowers on pedicels of 12 to 14 mm., the bracts large, foliaceous, ovate, 12–24 mm. long, 8–12 mm. wide; calyx of thick texture, pubescent, large, crateriform, 18–24 mm. long, divided to the middle into large foliaceous ovate-obtuse lobes of 8–10 mm. in width; corolla little exserted, slightly curved, pubescent.

MOLOKAI: Pali of Wailau and Pelekunu, July 1870, Hillebrand in herb. Berlin and Gray Herbarium; at the foot of a waterfall near Kamoku, flowering March 1910, Rock. no. 6118, in herb. College of Hawaii.

The single sheet of this species in the Berlin Herbarium bears the following legend:—"*Cyrtandra Pickeringii ?* A. Gray." The specific name *Pickeringii* was crossed out by Hillebrand and the name *macrostegia* was substituted for it. He also states: "*calyx corolla incurva paulla breviore, bracteis late foliaceis—trunco arboreo.* July 1870."

Cyrtandra macrocalyx is a distinct species, but Hillebrand's description of it is not quite correct, or rather the dimensions of leaf, petiole, peduncle, and pedicels are not the maximum dimensions actually occurring. The following dimensions may be supplied from a large series of specimens of this species: leaves up to 15 cm. long, serrulate to coarsely serrate, petioles up to 3.5 cm., peduncles up to 2.5 cm., pedicels up to 34 mm.; the texture of the calyx is thin rather than thick. The species is the common form on Molokai, where it grows on the exposed open ridges as well as in shaded ravines along stream beds. Those found in open places have the leaves more rounded and covered with a fulvous tomentum than those found in shady places; those of the latter locality have the leaves long and acuminate, of a fleshy texture, and of a greener color; the flowers are also larger.

Cyrtandra umbracculiflora Rock n. sp.

A stout shrub 3 m. high, the stems stout, with thick nodes, pubescent throughout; leaves (opposite?) alternate, large, ovate, acute at the apex, uneven-sided at the base, each side with a distinct sinus, the petiole apparently branching at the apex and forming a sinus on each side, 25 cm. long, 18 cm. wide, thick-chartaceous, the margin irregularly crenate to sinuately notched, the lobes with callous teeth at irregular intervals, hirtellous above, tomentose beneath with yellowish-gray hairs, especially along the stout midrib and veins; petiole clasping, stout, 10–15 cm. long, densely yellowish-tomentose; inflorescence yellowish-tomentose throughout, subumbellate, opposite to alternate, arranged along the stem, the common peduncle stout, flattened, 4.5 cm. long, 4 mm. broad, thickening and expanding subflabellately into two short thickened rays 5 mm. in length, each dividing again, pedicels of about even length, 13–15 in number, 3 cm. long; bracts subfoliaceous, lanceolate, acute to acuminate, 2.5 cm. long, 1 cm. broad; calyx irregularly lobed to near the base into broad, ovate-lanceolate segments acute at the apex, contracted below, indistinctly nerved, 12–15 mm. long, 5–7 mm. wide, hirtellous to tomentose inside and outside, corolla unknown; fruit ovate-oblong, rounded at the apex, crowned by two-thirds of the style, the latter articulate in the last (upper) third; stigmatic lobes broadly ovate, with the margins reflexed.

KAUAI: Forests of Kaholuamano along Waiakealoha stream, also Waialae

Valley along stream bed, 3,500 feet elevation, in company with *Cyrtandra Wawrai* fruiting Sept. 1909, Rock no. 5961 (type), in herb. College of Hawaii (Plate V).

Cyrtandra umbracculiflora is one of the most distinct new species of Cyrtandra found in the Hawaiian Archipelago. It is remarkable for its umbellate inflorescence and peculiar leaf bases. As already remarked, Kauai possesses the most distinct species of Cyrtandreae as well as of Lobelioideae. The same can be said of nearly all the other native plants of that island. It is somewhat difficult to place this species, as it is eligible for both sections Crotonocalyces and Schizocalyces. The writer has decided to place it in the latter section on account of the deep division of the calyx, notwithstanding the very broad lobes which in one or two instances seem to be united on one side, thus forming a very broad lobe with three short triangular teeth. The large leaves would also place it with Section Crotonocalyces. In habit it resembles *C. Wawrai*, but is otherwise quite different.

SECTION FOUR. CHAETOCALYCES HILLEBR. FL. HAW. ISL. 326. 1888

Calyx deeply split into linear or subulate lobes. Flowers mostly subumbellately arranged. Leaves thin, glabrous or hispid, large, ovate to oblong, decurrent at the base or acute at both ends.

The section Chaetocalyces, which Hillebrand still further designates as "*virides*," comprises the following species: *Cyrtandra Menziesii*, *C. kalichii*, *C. waiolani*, *C. gracilis*, *C. Macraei*, and *C. Lydgatei*. The following varieties may be recorded: *C. kalichii* var. *tristis*, *C. Macraei* var. *parvula*, *C. gracilis* var. *subumbellata*, and doubtfully *C. Menziesii* var. *Gaudichaudiana*. While the other sections furnished a number of new species and still more varieties, this section furnished only one new variety. It would appear that these species are more settled than are those of other sections occurring on the more centrally located islands of the group; of the species described in this section four occur on the island of Oahu, only one on Molokai and West Maui, and one on Hawaii. Section Microcalyces is closely connected with Section Chaetocalyces, and the majority of the species of the former occur also on Oahu.

C. tristis Hillebr. ms., which was described by Clarke, has been reduced to a variety of *C. kalichii* Wawra. Hillebrand united it with the latter and quoted his manuscript name as a synonym but not as a species. Clarke even placed it in a separate section. It took careful study to distinguish the two plants from dried material. None of the species belonging to this section have as yet been found on Kauai, the oldest island of the group.

CYRTANDRA MENZIESII Hook. & Arn. Bot. Beechey Voy. 91. 1841

Cyrtandra Brighami C. B. Clarke in DC. Monogr. Phan. 5: 221. 1883–1887

Branches obscurely quadrangular, the young shoots densely ferruginous-pilose at the apex; leaves quaternate, oblong, acuminate at both ends, subdenticulate to serrate, 9 cm. long, 3 cm. broad, scaberulous above, yellowish-pubescent beneath or almost glabrate; petiole 3 cm. long; peduncle 10–15 mm. long; pedicels six to fifteen, umbellately arranged, 10–15 mm. long; calycine lobes linear or subulate, somewhat villous; corolla straight, pubescent; fruit ovoid, glabrous.

INSULIS SANDWICH: Menzies in the Kew Herbarium, Menzies et Nelson in herb. British Museum. Gaudichaud no. 851 Voyage Bonite, in herb. Berlin and Gray Herbarium, and herb. College of Hawaii.

HAWAII: Woods of Kona, Hillebrand in herb. Berlin, and herb. College of Hawaii; Mann and Brigham no. 310, in Gray Herbarium and herb. Cornell University (Plate VI).

The writer is familiar with this species only from herbarium specimens. His own species *C. kohalae* comes very close to it. Clarke's *C. Brighami* cannot very well be separated from *C. Menziesii* Hook. & Arn. There are only very slight differences such as texture of leaves, slightly shorter peduncles, etc. The main features are exactly the same. Clarke states that it differs from *C. Menziesii* Hook. & Arn. primarily in the larger fruits which even in a young state do not resemble those of *C. Menziesii.* Hawaiian plants are extremely variable, and different-sized fruits may be found on a single Cyrtandra bush. Clarke very briefly describes or rather mentions a var. *β Gaudichaudiana* "with leaves always opposite and never whorled, mature fruit 8 mm. long, ellipsoidal, not exceeding the calyx." It was collected by Gaudichaud and specimens are to be found in the herbaria of De Candolle and Delessert; according to Clarke it was found together with *C. paludosa* and was perhaps confused with it by Gaudichaud. Hillebrand reports: "My specimens exhibit both opposite, ternate, and quaternate leaves, while in those collected by Menzies, Gaudichaud, and Mann they were quaternate." The variety *β Gaudichaudiana* of Clarke may be identical with Hillebrand's specimens with opposite leaves.

Gaudichaud's no. 851, Voyage Bonite, of which specimens may be found in the Berlin and Gray herbaria, is referred by Clarke to *C. Brighami.* The Gaudichaud specimen in the Gray Herbarium was identified by Asa Gray as *C. Menziesii* and marked as such in his own handwriting.

Mann and Brigham's no. 310, which served as the type of *C. Brighami* C. B. Clarke, and of which a specimen is in the Gray Herbarium, was also identified by Asa Gray as *C. Menziesii.* The type is in the Kew Herbarium.

CYRTANDRA KALICHII Wawra Flora **30**: 564. 1872

A shrub 2 m. high, simple, erect; leaves broadly ovate, membranous, acute at the apex, rounded at the base, *decurrent to half the length of the petiole,* rough hispid above, ferruginous-pubescent beneath, 3 dm. long, 18 cm. wide, coarsely dentate, the teeth again serrulate; petioles about 10 cm., *naked, not winged* in the lower 5–6 cm., winged or the leaf decurrent along the upper 4–5 cm. of petiole; peduncle axillary, this and the pedicels, bracts, and calyx hirsute with yellowish hairs; peduncles 12 mm., 3–5-

flowered; bracts about 6 mm., acute; pedicels 10–12 mm., the lateral ones shorter; calyx 7–10 mm., including the linear lobes, the latter more or less spreading; corolla hirsute outside toward the apex; the tube slender, exserted, nearly twice as long as the calyx, ampliate at the throat; ovary glabrous but hirtellous at the base of the style, the latter reddish-hirsute.

OAHU: "*Felsschluchten des Kalichithals,*" Wawra no. 1788 in herb. Vienna, clastotype in herb. College of Hawaii; Hillebrand, in herb. Berlin (included with *C. tristis*); Punaluu Mts., Koolau range elevation 2000 feet, flowering Nov. 14–21, 1908, Rock no. 931; same locality Dec. 3–14, 1908, no. 152, and Oct. 31, 1914, no. 1759, in herb. College of Hawaii; Wahiawa, Kaukonahua gulch, flowering May 15, 1909, Rock and Hosmer no. 3028, in herb. College of Hawaii.

Clarke was certainly a keen systematist; he correctly separated *Cyrtandra kalichii* Wawra occurring in the western range from plants growing at much lower elevations in the eastern range, for which plants he adopted Hillebrand's manuscript name *C. tristis*. Hillebrand classed both of these plants together and records his *C. tristis* as a synonym of *C. kalichii*. There are certainly decided differences between these plants; the most striking is in the petiole, which in *C. kalichii* is winged, or the leaf is decurrent about to the middle of the petiole, which is then naked. In *C. tristis* the petiole is much stouter and shorter and is broadly winged to the clasping base. The corolla tube is nearly twice the length of the calyx and is narrow and slender and also exserted; in *C. tristis* the corolla tube is not exserted; in fact the whole corolla is almost included, only the spreading lobes being free. In *C. kalichii* the calycine lobes are spreading, while in *C. tristis* the calyx is cylindrical, with the lobes erect. The leaves of *C. kalichii* are ovate to suborbicular, the margins are coarsely dentate and the teeth again serrulate; in *C. tristis* the leaves are obovate to ovate-oblong, and the margin is denticulate only. However, the plants are very closely related, and it is perhaps better to report Clarke's *C. tristis* as a variety of *C. kalichii* Wawra. Hillebrand writes *C. Kalihii* instead of *Kalichii* as Wawra spells it; the former spelling is correct. Wawra identified the aspirated Hawaiian *h* with the German *ch*, hence his improper spelling.

CYRTANDRA KALICHII **tristis** (Hillebr.) Rock n. name

Cyrtandra tristis Hillebr. ms. in C. B. Clarke, DC. Monogr. Phan. **5**: 227. 1883–1887.

Cyrtandra Kalihii Hillebr. (not Wawra) Fl. Haw. Isl. 334. 1888 (in part).

A sparingly branching shrub, the stems fleshy, stout, subterete; leaves large, ovate-oblong, denticulate, up to 45 cm. long and 20 cm. broad including the short winged fleshy clasping petiole; cymes many-flowered; peduncles about 6 mm., these and the whole inflorescence hirsute with dark reddish-brown hairs; pedicels of variable length; calyx cylindrical, the linear lobes erect and as long as the corolla; ovary glabrous; style puberulous, stigma of two elongate lamellae.

Oahu: In deep, gloomy ravines, Hillebrand in herb. Berlin and Gray Herbarium, and Kew; Mt. Kaala, Hillebrand, in herb. Berlin and College of Hawaii; Mt. Olympus trail, dense shade, flowering Sept. 1917, Rock, in herb. College of Hawaii; deep ravines of Palolo Valley along stream beds and waterfalls, flowering February 9, 1918, Rock and Crawford no. 13081, in herb. College of Hawaii.

The plant in question is certainly distinct enough at least to be classed as a variety of *C. kalichii.* Clarke described it as a species and even classed it in a different section from *C. kalichii.* For further consideration of the specific or varietal merit of this plant see the discussion under *C. kalichii.*

Cyrtandra waiolani Wawra Flora **30**: 566. 1872

Cyrtandra hirsuta Hillebr. ms. not Jack.
?*Cyrtandra Hillebrandi* Vatke ms. not Oliver ms.
Cyrtandra lasiodon C. B. Clarke ms.
Cyrtandra oahuensis Lév. in Fedde Repert. Sp. Nov. **10**: 124. 1912

A small tree with slender branches; leaves elliptical, acuminate at both ends, thin herbaceous, hirsute above with multicellular hairs, sparingly pubescent beneath but strongly hirsute on ribs and veins; 7–12 cm. long, 2.5–4.5 cm. wide, on petioles of 1.5–2.5 cm.; peduncles one-flowered of the length of the petiole, or 2-flowered and the pedicels half the length of the peduncle; bracts linear, 1 cm.; calyx campanulate, 1.5 cm., deeply split into linear, hirsute lobes which are recurved at the apex when with fruit; corolla glabrous at base, hirsute at the constricted throat and below the lobes, glabrous within; ovary glabrous; style hairy, articulate at the base, deeply bilamellate; fruit large, ovoid-ellipsoidal, apiculate.

Oahu: "*Waldschluchten des Waiolani,*" Wawra no. 1792, in herb. Vienna, and part of type in herb. College of Hawaii; gulches of Konahuanui, Kalihi, Moanalua, Kaala, Hillebrand, in herb. Berlin, herb. College of Hawaii, and Gray Herbarium; Punaluu Mts., Koolau range, flowering Aug. 1908, Rock, in herb. College of Hawaii; same locality, Nov. 14–21, 1908, Rock no. 884, in herb. College of Hawaii; Punaluu, May 1910, Faurie (as *C. oahuensis*), co-type in herb. College of Hawaii.

The species is by no means confined to Mt. Waiolani, but occurs all along the western range of the Koolau mountain chain. The specimens from the Punaluu mountains are a little less hairy and occasionally the calycine lobes are a little wider, which fact perhaps persuaded Léveillé to describe it as a new species.

Cyrtandra gracilis Hillebr.; C. B. Clarke, Monogr. Phan. **5**: 226. 1883–1887; Hillebr. Fl. Haw. Isl. 333. 1888

A shrub 2.5–3.5 m. high, the angular branches slender, faintly pruinose, those and the cyme soon glabrate, leaves opposite, membranous, green on both faces, paler beneath, puberulous along the veins or thinly pubescent over the whole lower surface, glabrous above, ovate or elliptical-oblong, 10–20 cm. long, 5–10 cm. wide, on petioles of 2.5–8 cm., caudately acuminate at the apex, serrulate, suddenly contracting and shortly decurrent; flowers generally three or five to seven, on a common peduncle of about 15 mm.; pedicels slender, 2–5 cm., thickening above; bracts linear-lanceolate, 6–12 mm.; calyx glabrate, split to the base into linear lobes 12–18 mm. in length, shorter than, or equaling, the corolla; corolla pruinose, slightly curved or suberect, 25 mm. long, ampliate at the throat; ovary glabrous, elliptical, the style hirtellous, slender; stigmatic lobes obovate, spreading; mature fruit 22 mm. long, 5 mm. broad, narrow-oblong, acute, sessile.

OAHU: near Palolo (on both mountain ranges), not uncommon in Nuuanu; Konahuanui gulch, Hillebrand, in herb. Kew and Berlin and Gray Herbarium, co-type in herb. College of Hawaii; Punaluu Mts., Koolau range, along stream beds, flowering Dec. 3–14, 1908, Rock nos. 325, 7421, 762, in herb. College of Hawaii (Plate VII).

Cyrtandra gracilis is somewhat variable; the specimens from the Punaluu mountains have a stout quadrangular stem and not a slender one, and the leaves are larger and have a close fenestrate venation; the petioles are also stouter, and the peduncles are shorter. Hillebrand states "flowers generally three"; his specimens have nearly all from six to seven flowers; the calycine lobes which, he remarks, are generally longer than the corolla, are usually only one half the length of it. His variety *subumbellata* seems to be only a large form of the species, but owing to its extraordinary size is here retained as a variety.

The specimen in the Gray Herbarium has the leaves more elliptical-oblong and the exceedingly long pedicels measuring over 5 cm., while the peduncles vary from 1 to 2 cm.

The long slender calycine lobes exceed the fruit, but do not quite equal the flowers in length.

CYRTANDRA GRACILIS SUBUMBELLATA Hillebr. Fl. Haw. Isl. 334. 1888

Habit of the species; branches stout quadrangular, with stout nodes at the places of leaf attachment; leaves large, broadly ovate, caudately acuminate at the apex, decurrent at the base for a short distance, 22–31 cm. long, 10–18 cm. wide, thin membranous, chartaceous, dark green above, grayish-puberulous underneath, with prominent nerves; margins broadly dentate excepting the base; petiole 3.5–7 cm. long; inflorescence axillary, extending down the stem; the common peduncle 1.5 cm.; flowers six to eight on slender pedicels of 12–25 mm.; bracts numerous, linear, up to 17 mm. long; calyx as in the species, the lobes linear, as long as the corolla or shorter; corolla smaller, 16–20 mm. long, pruinose.

OAHU: Hillebrand; in dense forest along Kaliuwaa stream in the mountains above Punaluu, above second waterfall, flowering Dec. 24–28, 1908, Rock no. 416, in herb. College of Hawaii; same locality, Oct. 31, 1914, Rock no. 13078, in herb. College of Hawaii.

There is no specimen extant in the Berlin Herbarium of Hillebrand's collection. There is no doubt that the writer's plant is identical with the description. It is larger in every way than the species, the leaves are certainly the size of *C. Macraei* and even larger, but the calycine lobes are long and linear to almost subulate. It is an intermediate between *C. Macraei* and *C. gracilis*, or *C. gracilis* is a variable species with larger leaves in shaded situations and smaller ones in open or exposed forest ridges.

CYRTANDRA MACRAEI A. Gray. Proc. Amer. Acad. **5**: 352. 1862

A shrub about 3 m. high or more, the branches strictly quadrangular, subherbaceous, green, with a pruinose efflorescence towards the apex; leaves broadly ovate, opposite, 15–22 cm. long, 10–15 cm. wide, acuminate at the apex, rounded or slightly decurrent at the base, thin-membranous, glabrate above, canescent underneath with a faint pubescence, the margin dentate to the base; petiole stout, pruinose, 5–14 cm. long; inflorescence a subumbellate, pruinose cyme, axillary in the upper leaves and extending some distance down the stem, in the fleshy ascending branches axillary in the lowest leaves; flowers numerous, ten to twenty, on a common peduncle of 5–6 mm., the latter branching into, or bearing at its apex, three rays, each bearing a number of slender pedicels of variable length, those with fertile flowers reaching a length of 8 mm.; calyx parted to near the base into acute or acuminate, lanceolate lobes 2–3 mm. in length; corolla tube of even width, slender, curved, 12 mm. long, 3 mm. wide, with small pruinose lobes; ovary pruinose, elliptical; style articulate at or below the middle; fruit oblong, rounded at both ends, 12 mm. long, 7 mm. wide when fully mature.

OAHU: Macrae, May 1825 (type), in Gray Herbarium; Gaudichaud, Voyage Bonite; Brackenridge, U. S. Explor. Exped. (*teste* Gray); eastern division of main range, Niu to Wailupe Valley, Hillebrand no. 325, in herb. Kew and Berlin, part of specimen (*ex* Wailupe V.) in herb. College of Hawaii; Mann and Brigham, without number, in herb. Cornell University; small ravine of Palolo Valley along stream bed, flowering February 9, 1918, Rock no. 13077, in herb. College of Hawaii (Plate VIII).

Cyrtandra Macraei is exceedingly close to *C. gracilis*, but differs from it in the short calycine lobes, shorter corolla, and numerous flowers.

CYRTANDRA MACRAEI **parvula** Rock n. var.

A small tree or shrub 3 m. high, with numerous slender, angular branches, pruinose towards the apex; leaves ovate-oblong, caudately acuminate at the apex, cuneate at the base, bright green and puberulous along the nerves and leaf-base above, grayish to canescent below, and pubescent, especially along the midrib and nerves, thin-chartaceous, denticulate excepting the base, 10–15 cm. long, 5.5–8 cm. wide, on petioles of 3.5–6.5 cm.; inflorescence densely crowded in the axils of the upper leaves, pruinose, the peduncle 3–4 mm.; pedicels 5 mm.; flowers numerous, glomerate; calyx 3 mm., including the lanceolate lobes; corolla 8 mm , white-pruinose in the bud, little less so when mature, berry elliptical, acute, 6–8 mm. long.

OAHU: In deep ravines of Palolo Valley, along water-courses, flowering and fruiting February 9, 1918, Rock no. 13079 (type), in herb. College of Hawaii.

This variety differs considerably from the species; mainly in the smaller leaves and dense agglomerate inflorescence which is confined to the upper leaf axils; the flowers are much smaller, as are the fruits.

CYRTANDRA LYDGATEI Hillebr. Fl. Haw. Isl. 335. 1888

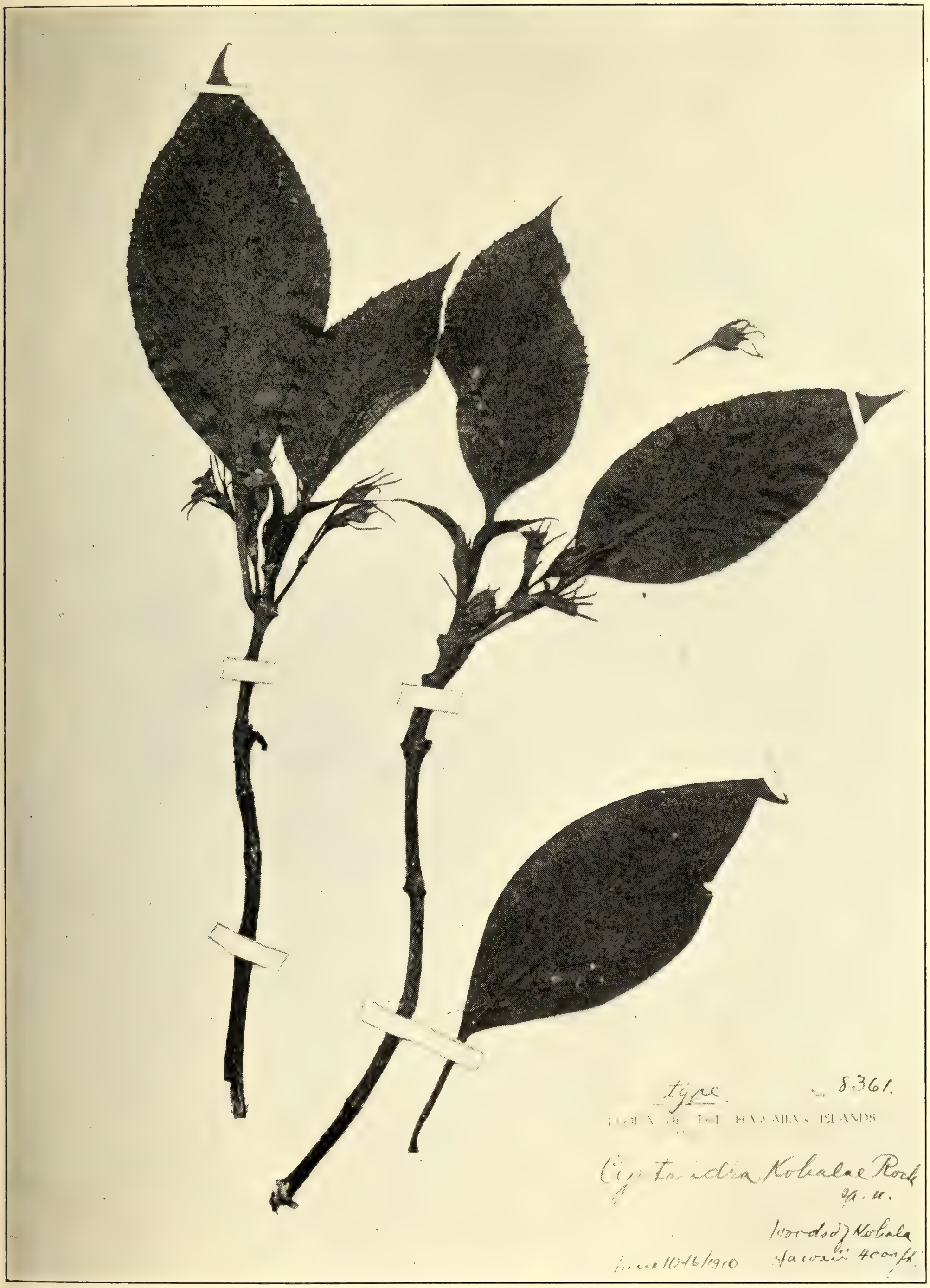
A small tree 4–6.5 m. high, branches villous with pale ochraceous hairs; leaves opposite, thin, flaccid, green on both sides, sparingly hispid with multicellular hairs above, puberulous along rib and nerves beneath, pubescent towards the base, broadly ovate or suborbicular, 10 to 22 cm. long, 6–14 cm. wide, shortly acuminate at the apex, rounded or cuneate-decurrent, somewhat uneven-sided, at the base; petiole not margined, 3–10 cm. long; flowers two to seven, subumbellately arranged on a peduncle of 1.5 cm., the pedicels 1–1.5 cm.; bracts narrow-lanceolate, thin, 6–8 mm.; calyx green, hirsute, cylindrical, divided to near the base into linear-acute to spathulate lobes; corolla slightly exserted, hirsute, 12–14 mm., the tube narrow, curved, the lobes large, spreading; ovary glabrous, with broadly lamellate style; fruit broad, ovoid, 15 mm. long.

MOLOKAI: In deep ravines of Kalae and Mapulehu, Lydgate, in herb. Berlin and Gray Herbarium; Mapulehu Valley, fruiting April 1910, Rock, in herb. College of Hawaii; Halawa Valley, fruiting April 1910, Rock, in herb. College of Hawaii; Kalae, in deep gulches, flowering May 1918, Rock and Dunbar no. 14064, in herb. College of Hawaii.

MAUI: Gulch of Honokawai, Kaanapali, West Maui, Aug. 1870, Hillebrand, in herb. Berlin and Gray Herbarium, and part of type in herb. College of Hawaii; Honomanu gulch, northeastern slope of Haleakala, East Maui, flowering May 1911, Rock, in herb. College of Hawaii.

The specimens from Maui, especially East Maui, have the calyx lobes decidedly spathulate, while those from Molokai have the calycine lobes linear-lanceolate, acute. The leaves are somewhat larger than the dimensions given by Hillebrand. A distinct species which comes close to *C. lysiosepala*, save that the calyx lobes are not reflexed and the calyx remains cylindrical in shape, the leaves are very much larger, and the inflorescence is up to 7-flowered, subumbellate.

COLLEGE OF HAWAII, HONOLULU.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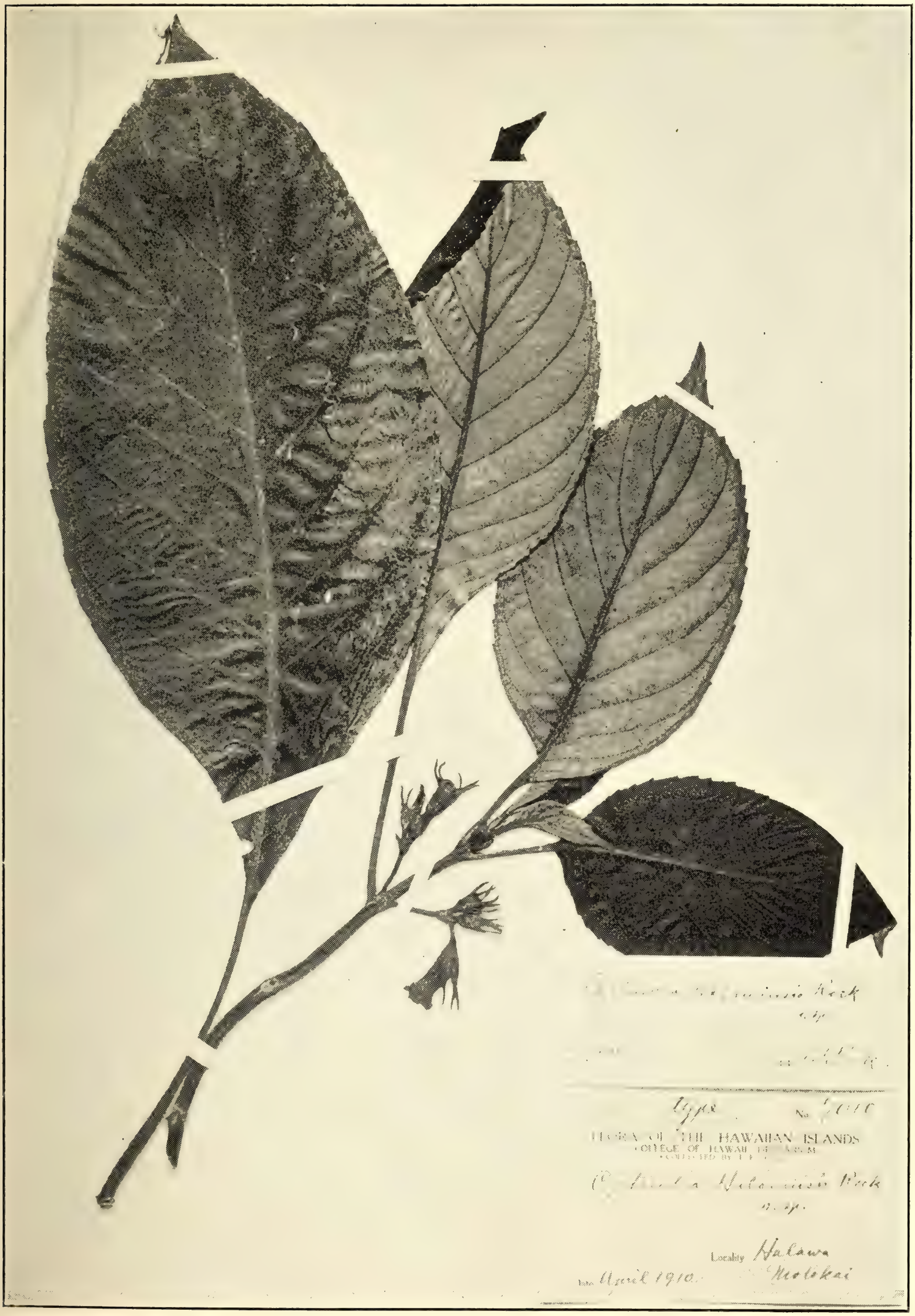
8361.
FLORA OF THE HAWAIIAN ISLANDS
Cyrtandra Kohalae Rock
sp. n.
(woods) Kohala
Hawaii 4000 ft.
June 10-16/1910

ROCK: TYPE OF CYRTANDRA KOHALAE ROCK.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Type No.
FLORA OF THE HAWAIIAN ISLANDS
COLLEGE OF HAWAII HERBARIUM
Rock
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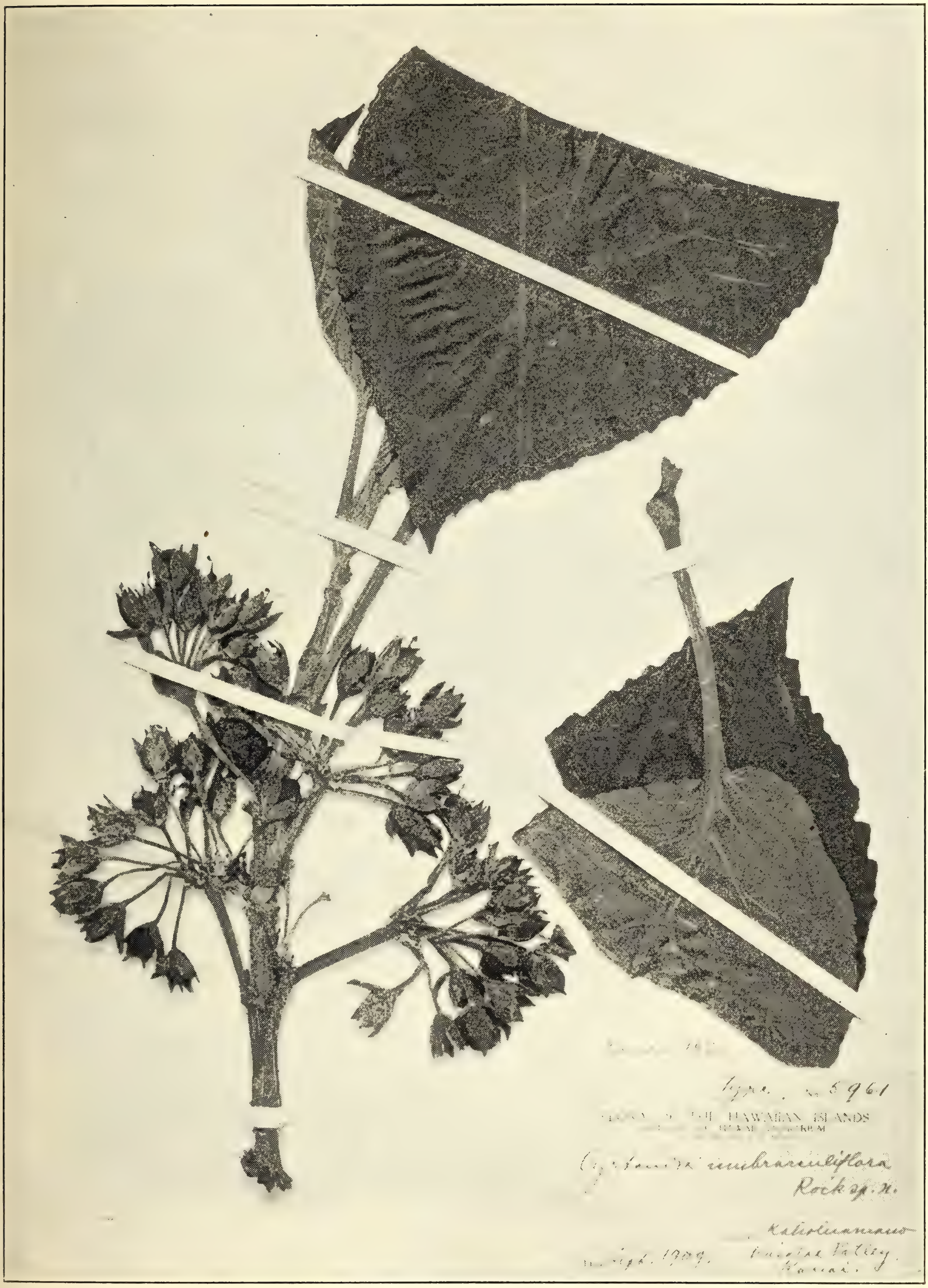
Locality Halawa Molokai
April 1910.

ROCK: TYPE OF CYRTANDRA HALAWENSIS ROCK.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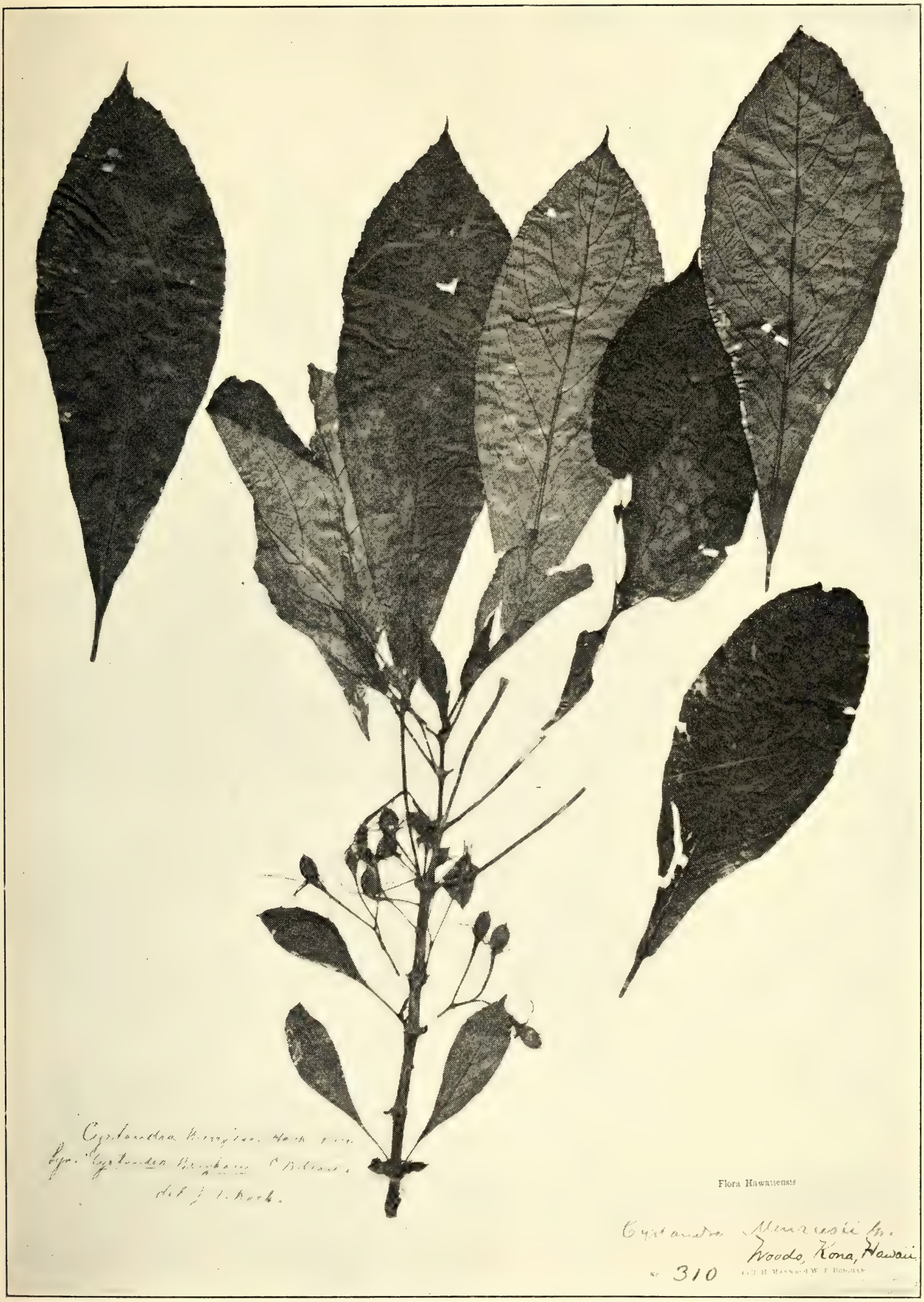
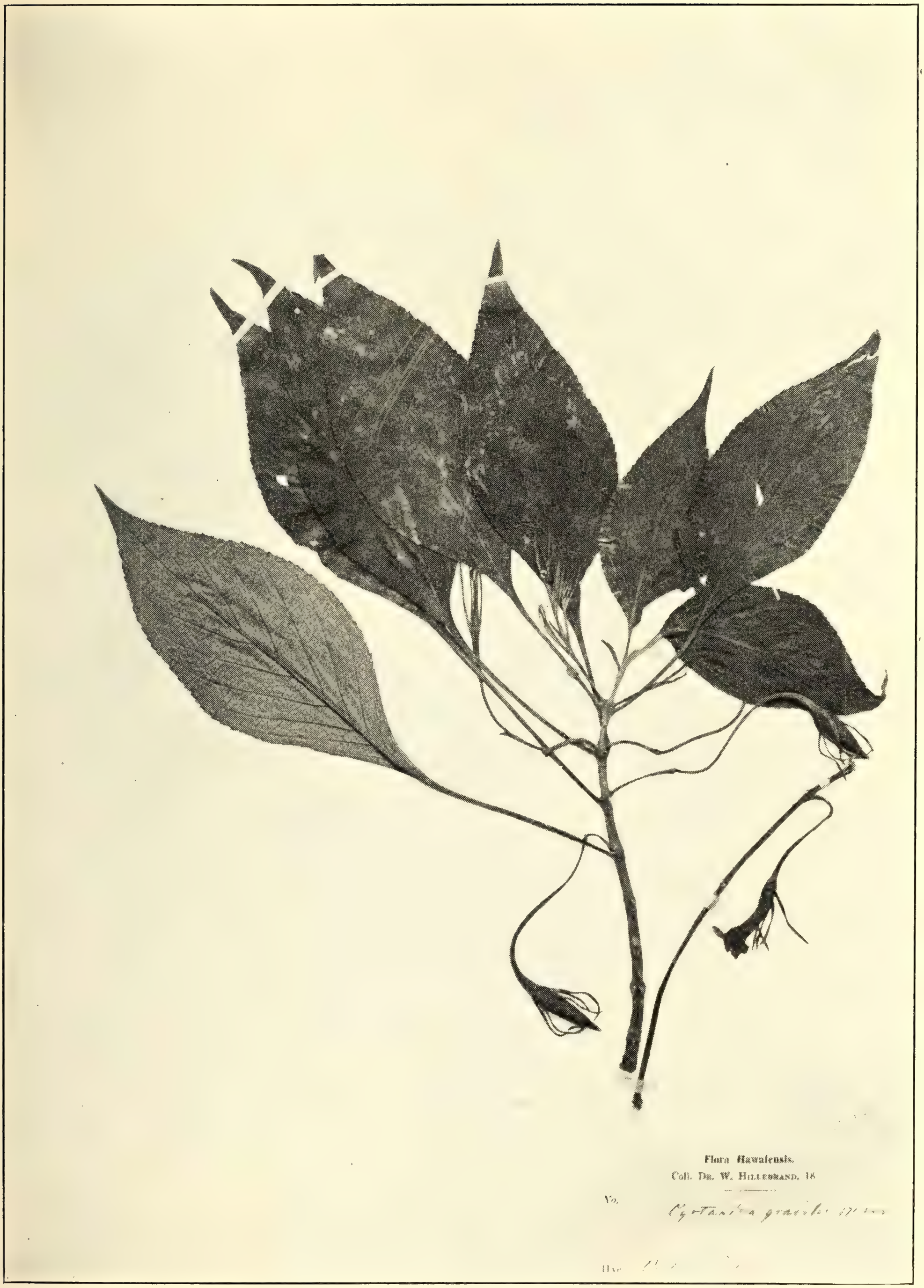
HAWAIIAN ISLANDS
Cyrtandra umbracculiflora
Rock sp. n.
Sept. 1909.

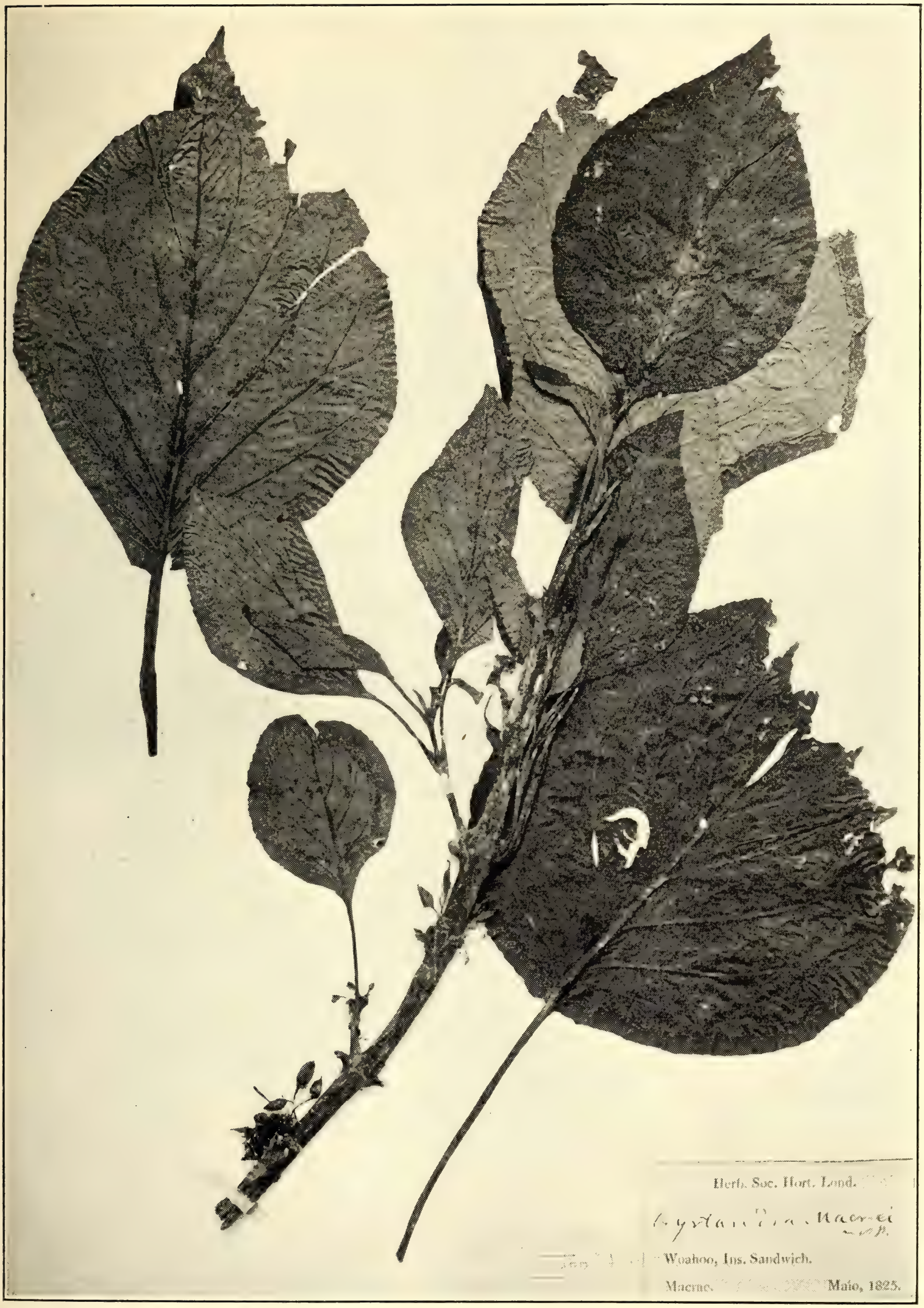
ROCK: TYPE OF CYRTANDRA UMBRACCULIFLORA ROCK.


Flora Hawaiiensis
Cyrtandra Menziesii H.
Woods, Kona, Hawaii.
310

ROCK: CYRTANDRA MENZIESII HOOK. & ARN.


Flora Hawaiensis.
Coll. Dr. W. Hillebrand. 18
No.
Cyrtandra gracilis
Hab.

ROCK: CYRTANDRA GRACILIS HILLEBR.


Herb. Soc. Hort. Lond.
Cyrtandra Macraei
Woahoo, Ins. Sandwich.
Macrae. Maio, 1825.

ROCK: TYPE OF CYRTANDRA MACRAEI A. GRAY.

THE ECOLOGIC FOLIAR ANATOMY OF SOME PLANTS OF A PRAIRIE PROVINCE IN CENTRAL IOWA

Ada Hayden

Introduction

While exact records of environmental factors such as edaphic features, temperature, light, water, and biotic relationships are essential in the determination of the character of plant habitats, such data serve merely as an introduction to the investigation of the adaptation or equipment of plants for living in specific locations. While certain external characteristics, such as small or dissected leaves, are associated with sun plants, and broad leaves with shade plants, histological study reveals in greater detail any modifications of the normal type of tissues which are known to perform special activities. That the activities pertaining to the life processes of the plant are closely associated with its use of water is a well known physiological fact, so that not only the available water of the habitat but that which the plant actually uses or what passes through it in the transpiration stream is an important indicator of its toleration of conditions peculiar to its particular habitat. Work has been done by Livingston, Bakke, Shreve, and others on the transpiration of plants, and Bakke (1) has proposed a classification of plants as xerophytes, mesophytes, or hydrophytes on the basis of transpiration, which it seems is a very exact indicator of the available water used. While indices of transpiration would no doubt be desirable in connection with a morphological study, and would probably throw some light on whether a special type of tissue were characteristic of a species because of reaction to habitat or because of ancestral influence, the observations reported in this paper are confined to the morphological phase alone.

Selection of Material

Leaves and subterranean portions were chosen for examination since these organs are critical "indicators" of absorption and transpiration of water and therefore are more closely related to the regulation of the water supply than stems, which serve primarily as conductors. The plants selected were representative species of their habitats, *i. e.*, those species which were facies, or prominent in frequency or as to their vegetative perfection, for such plants may be regarded as instances of successful occupation of their respective environments.

METHODS

Free-hand sections were made from typical mature leaves, usually selected from different plants, and from these the more representative sections were selected for mounts. After cutting, the sections were killed and fixed by immersion in hot alcohol, stained with a water-soluble safranin followed by haematoxylin, cleared in cedar oil and clove oil, and mounted in Canada balsam. Drawings of the leaves were made by camera lucida to a single scale of magnification. *Qualitative data have been sought rather than quantitative.* The thickness of the epidermis of leaves of one species as compared with the thickness of epidermis of another is not necessarily a positive or negative indicator of xerophytism, for one leaf may have trichomes plus a degree of thickness of epidermis found in the trichomeless leaf. Data concerning features regarded as "indicators" have been collected and relative proportions of tissues have been noted in individual plants.

HISTORICAL

While many physiologists are yet skeptical concerning the use of such terms as "reaction" of a plant to its habitat, "modification," or "adaptation," careful investigation has shown that species do change their structure under different environmental conditions, although these words have been somewhat inexactly used and perhaps more exact terms might be evolved. Whether certain histological characters are due to environment or to ancestral influence can be determined only by some histological knowledge of closely allied forms and some experimental evidence regarding one species placed under different conditions. Schimper (15), who has done extensive research of recognized excellence in histology, ecology, and plant geography, as well as some work in physiology, states: "All experiments have led to essentially similar results. External conditions which, either by diminishing the absorption of water or by accelerating its exit from the plant, disturb the equilibrium in a sense hostile to the plant, occasion, as a rule, the following deviations from normal structure: (1) Reduction of surface, the volume being assumed constant. (2) Diminution of intercellular spaces containing air. (3) Augmentation of vessels and sclerenchyma. (4) Lengthening of the palisade cells, frequent but not universal. (5) Increase in the thickness and amount of cutin of the outer wall of the epidermis. (6) Sinking of stomata. (7) Increased number of air-containing cells. (8) Supply of water-storing cells, such as double epidermis, aqueous tissue, mucilage cells.

Haberlandt (6) calls attention to two main principles of the anatomical structure of the photosynthetic system: (*a*) the principle of maximum exposure of surface, and (*b*) the principle of expeditious translocation.

The morphological structure of photosynthetic cells whose modifications are regarded as facilitating translocation are summarized by Haberlandt under three systems of structure. In system 1, the photosynthetic tissue is itself responsible for the removal of synthetic products from the entire organ. In system 2, distinct tissues are set apart for photosynthesis and for translocation, the synthetic products being transferred directly from one to the other. In the third and most efficient system, the photosynthetic products are not transferred directly from photosynthetic elements to the different channels but pass first through special intermediary tissues.

Sachs has shown by experiment that starch is quickly removed from specialized parenchyma cells. Haberlandt has observed that the girdle type of palisade shows a very rapid elimination of starch.

There is apparently diversity of views regarding development of palisade parenchyma, some of which Miss Starr (18) in her study of the anatomy of dune plants summarized as follows: "Mrs. Clements (4) considered light the principal factor in the development of deep palisade. . . . Wagner reported that Alpine plants exposed to decreased transpiration did not show a reduction in palisade, and concluded that not transpiration but assimilation was more effective in producing that tissue. Pick (14) thought the elongated form of the palisade is ancestral, but that for a strong development light is necessary; Dufour (5) agreed with him in this respect. Stahl related palisade development to light. Eberdt thought increase in palisade development is caused by assimilation and transpiration working together, and that light in itself is never the cause that calls forth palisade parenchyma. Vesque and Viet (20) concluded from their experiments that light and dry air (accelerating transpiration) result in a greater development of palisade. Bonnier (3) adds temperature considerations to these two factors. Kearney (9) considers excessive transpiration accountable for both increased palisade and succulency. Heinricher related equilateral structure to the vertical position of leaves and thought it due to sunny and dry situations, dryness being secondary to strong illumination, as some plants growing in damp situations have equilateral leaves."

Solereder (19), who has compiled two comprehensive volumes on the systematic anatomy of the dicotyledons in which an enormous amount of data has been correlated, substantiates Schimper's views as to the anatomical indicators of xerophytism, and the reverse, but adds concerning the determination of adaptive features that these can be ascertained only by (1) examination of individuals of the same species from different habitats; (2) experimental treatment under definite conditions differing from those of the natural habitat; (3) the study of a larger group of plants undertaken in relation to the geographical area over which its members are distributed; and (4) comparative investigation. In speaking of the enumerated characters generally associated with xerophytic plants, such as thick cuticle, depressed stomata, etc., Solereder says that all these adaptations must not be supposed to be quite general, for if that were so, all plants which were subject to the same conditions would possess the same biological structural features even if they belonged to the most widely separated groups. This is only exceptionally true. Experience shows that one species reacts in one way, other species in a different way, under the action of the same stimulus, but that the reaction is often of the same kind in plants belonging to the same phylum. Thus one species protects itself against desiccation solely by means of mucilage receptacles, another by the development of hypodermal aqueous tissue, a third by enlargement of epidermal cells, and others by two or more of these features. Reiche, Volkens, and others have shown that climate and habitat do not impress any one definite type of anatomical structure upon all the species of a certain geographical area. Species possess a definite plasticity which, however, may vary in degree and direction in individuals; in such cases we may find discrepancies between structure and external conditions. According to Vesque and Areschoug the leaves of Nelumbium bear stomata on the upper side only, just like floating leaves. This fact is drawn from the theory that Nelumbium is derived from an ancestral form possessing floating leaves and is supported by the results of physiological researches in which it has again been emphatically shown that the anatomical structure is the product of two factors—adaptation and heredity. The second factor, which sometimes becomes more noticeable than the first, allows us to employ biological structural features to a very considerable extent for systematic purposes.

Biological characters serve principally for the diagnosis of species. Within the same group of affinity, these characters are often identical in all those forms in which they appear; or they may be constant for groups of allied species, for genera, or for small orders. Biological characters may be divided into those which differ qualitatively and those which differ quantitatively; of these the former have the greater systematic value. The presence of hypoderm in a leaf is a more important fact than the number of layers of hypoderm. Abundant material of the same species from different habitats and cultural conditions should be compared.

DESCRIPTION OF LEAVES

Gramineae

Andropogon scoparius Michx.

Habitat: Dry soil; hill crests; slopes.

[1] Orientation and arrangement: Blade ascending, appressed to stem when young; opposite.

Gross structure: Lanceolate; blade glabrant except near sheath.

Histology (fig. 1, plate IX):

Outer walls of epidermis twice as thick as inner; bulliform cells prominent.

Parenchyma: Palisade cells concentric around the vascular bundles; spongy tissue between bundles; vascular tissue prominent.

Stomata small.

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[1] The term *orientation* as here used refers to the plane in which the leaf blade lies, whether horizontal, vertical, or ascending.

Summary: The bulliform cells are prominent. The thickened outer walls of the epidermal cells are indications of water conservation. This plant not only grows in a dry habitat but has abbreviated roots.

Bouteloua curtipendula (Michx.) Torr.

Habitat: Dry hill crests and slopes.

Orientation and arrangement: Ascending; alternate.

Gross structure: Small lanceolate-linear; flat or involute; scabrous above; sometimes pubescent beneath.

Histology (fig. 2, plate IX):

Outer walls of epidermis about twice as thick as inner walls; upper epidermal cells terminating in barb-like points at intervals; lower epidermis with trichomes; bulliform cells prominent.

Mesophyll reduced, represented by a radial row of palisade cells around the bundles.

Vascular tissue prominent, including a row of large water-storing cells.

Stomata small.

Summary: The thickened epidermal cells prominent, bulliform cells well developed; the conspicuous vascular tissue and the reduced photosynthetic tissue are marked indicators of conservational facilities. Bouteloua lives in drier areas and has a more restricted habitat than *Andropogon scoparius*.

Muhlenbergia mexicana (L.) Trin.

Habitat: Damp soil; low land; alluvial basin.

Orientation and arrangement: Ascending; alternate; somewhat appressed to stem.

Gross structure: Lanceolate-linear; small; scabrous.

Histology: Homogeneous (fig. 3, plate IX).

Outer walls of epidermis slightly thicker than inner; cells terminating in barb occasionally; bulliform cells not prominent.

Parenchyma: Spongy in appearance; of roundish to oval cells compactly arranged.

Vascular tissue not prominent.

Stomata small.

Summary: This plant shows little tendency to conserve water. Vascular tissue is not so prominent as in grasses of drier habitats. Epidermis is not so specialized while photosynthetic tissue is fairly prominent.

Leersia oryzoides (L.) Sw.

Habitat: Alluvial basin; edge of swamp.

Orientation and arrangement: Ascending, somewhat appressed to stem; alternate.

Gross structure: Narrowly lanceolate; scabrous.

Histology: Homogeneous (fig. 4, plate IX).

Outer walls of epidermis slightly thickened; bulliform cells not prominent.

Vascular tissue fairly prominent.

Stomata small.

Parenchyma spongy; compact.

Summary: Water-conserving measures, though somewhat evident in the thickened outer wall of the epidermis and the differentiation of bulliform cells—are not so prominent as in *Andropogon scoparius* (fig. 4, plate IX) and *Bouteloua curtipendula* (fig. 2, plate IX), while photosynthetic tissue is better developed than it is in the leaves of the two last-named species.

Polygonaceae

Polygonum Muhlenbergii (Meisn.) Wats.

Habitat: Alluvial basin; wet soil and shallow water at edge of ponds. Not submerged.

Orientation and arrangement: Horizontal to ascending; petiolate.

Gross structure: Lanceolate to ovate; water form smooth; land form scabrous.

Histology: Bifacial (figs. 5*a* and 5*b*, plate IX).

Epidermis: Moderately thin-walled; slightly thicker on outer surface of water form; trichomes present on land form, also mucilaginous epidermal cells.

Stomata smaller on land than on water form.

Palisade parenchyma in two layers in both forms; the upper of irregular long cells, the lower of shorter and irregular cells; the land leaf is thicker than the water leaf, because the palisade parenchyma occupies 3/8 of the parenchyma space in the water form and 5/9 of the parenchyma space in the air form.

Spongy parenchyma about equally developed in both forms; loose.

Summary: These leaves indicate abundant water for needs with little provision for its conservation. The air leaf is a little thicker because of its increased thickness of the palisade, and its lower epidermis is equipped with trichomes and smaller stomata.

Ranunculaceae

Anemone cylindrica Gray.

Habitat: Dry, gravelly hill crests; dry, wind-swept.

Orientation and arrangement: Blade horizontal; radical leaves petiolate, involucrate leaves sessile.

Gross structure: Digitately cleft to parted; pubescent.

Histology: Bifacial (fig. 6, plate IX).

Epidermis with curved walls; outer walls about three times as thick as inner; large-celled compared with the parenchyma.

Stomata small; level with lower edge of epidermis.

Palisade parenchyma in 2 layers; slender; irregular; occupies 2/5 parenchyma space.

Spongy parenchyma small-celled; close; compact; about 3/5 the parenchyma space.

Summary: This leaf is equipped with trichomes, thick outer epidermal walls, and compact photosynthetic tissue.

Leguminosae

Amorpha canescens Pursh.

Habitat: Hill crests and dry hillsides.

Orientation and arrangement: Horizontal; petiolate; alternate.

Gross structure: Pinnately compound; hoary pubescent.

Histology: Subcentric (fig. 7, plate X).

Epidermis with approximately straight-sided cells; cells small; outer walls hardly thicker than the inner; trichomes present.

Palisade parenchyma in four layers graduated in size, longest on upper side; compact.

Spongy parenchyma absent.

Summary: The compact structure and the prominent trichomes show marked conservational features in accord with its specialized photosynthetic tissue. This plant, though living in dry situations, has a deep root which may reach a lower water table than the roots of some of its associates.

Baptisia leucantha T. & G.

Habitat: Alluvial basin; moist soil.

Orientation and arrangement: Horizontal or somewhat inclined; almost sessile.

Gross structure: Palmately 3-foliolate; leaflets wedge-shaped.

Histology: Subcentric (fig. 6*a*, plate X).

Walls of outer epidermis about three times as thick as inner; fairly large oblong cells with long axis horizontal.

Palisade parenchyma rather loose; cells medium-sized. Stomata slightly depressed.

Summary: The somewhat thickened outer wall of the epidermis and the slightly depressed stomata show some tendency to check transpiration, but the prominent palisade parenchyma denotes marked photosynthetic activity. This plant not only lives in a fairly moist habitat, but has deep roots, so that its water supply seems insured.

Rhamnaceae

Ceanothus americanus L.

Habitat: Dry, gravelly slopes.

Orientation and arrangement: Horizontal; alternate; short-petioled.

Gross structure: Ovate to oblong-ovate; somewhat pubescent.

Histology: Centric (fig. 8, plate X).

Epidermis: Thin-walled, the outer walls hardly thicker than the inner; cells small. Lower epidermis in scallops.

Stomata small; same plane with the lower epidermis.

Palisade parenchyma one layer on each side; lower layer rather irregular; occupies 2/3 parenchyma space.

Spongy parenchyma relatively large; compact; occupies 1/3 the parenchyma space.

Summary: While this plant lives in a dry, well-drained, exposed habitat, it does not show such protective characters as might be expected. Nothing is prominent except the compactness of structure. This may be accounted for by the fact that it has a relatively deep root which can reach a lower water table than the roots of some of its associates.

Umbelliferae

Eryngium yuccaefolium Michx.

Habitat: Dry soil; hill crests and slopes.

Orientation and arrangement: Ascending; stem leaves alternate; radical leaves whorled; sessile.

Gross structure: Ovate-lanceolate, cuspidate-tipped; rigid, spinose.

Histology: Centric (fig. 16, plate XI).

Epidermal outer wall 3 times as thick as inner wall; large-celled.

Palisade parenchyma one layer on each side; irregular in shape and arrangement; occupies 1/8 of parenchyma space; large-celled.

Spongy parenchyma cells elongated, their long axes at right angles to the palisade; large-celled.

Summary: Prominent large-celled photosynthetic tissue with prominent air space. Epidermal walls fairly well developed.

Primulaceae

Steironema lanceolata (Walt.) Gray.

Habitat: Alluvial basin; low, wet soil.

Orientation and arrangement: Horizontal to ascending; opposite; petioles graduated in length.

Gross structure: Lanceolate; glabrous.

Histology: Bifacial (fig. 9, plate X).

Epidermal cells large; horizontally oval; outer walls about twice as thick as inner.

Stomata slightly depressed.

Palisade parenchyma 1 layer; large, occupying 1/3 the parenchyma space.

Spongy parenchyma loose.

Summary: This leaf structure indicates the photosynthetic activity with slight conservational tendencies, as suggested by the slightly thickened outer epidermal walls and the depressed stomata.

Apocynaceae

Apocynum cannabinum L.

Habitat: Alluvial basin; wet soil.

Orientation and arrangement: Opposite, short-stemmed; horizontal to ascending.

Gross structure: Ovate to oblanceolate, glabrous or slightly pubescent.

Histology: Bifacial (fig. 10, plate X).

Upper and lower epidermis having thicker outer than inner walls. Lower epidermis scalloped, the walls in the middle of the scallops being 3 times as thick as the inner walls; outer walls curved.

Stomata small, slightly depressed; lower surface.

Palisade parenchyma 3 layers; slender; space occupied equal to that of spongy parenchyma.

Spongy parenchyma with moderate air space.

Summary: The thickenings of scallops seem practically equivalent to a uniformly thickened cuticle, for the thin places are opposite walls. This leaf shows indications of abundant water with adequate conservation facilities and a tendency to endure drought.

Asclepiadaceae

Asclepias verticillata L.

Habitat: Alluvial basin. Basal slopes or low, level, moist areas.

Orientation and arrangement: Whorled, somewhat appressed toward the stem in an upward direction.

Gross structure: Linear with revolute margins, glabrous.

Histology: Bifacial (fig. 11, plate X).

Upper epidermis twice as thick as lower, outer wall thicker than inner in both cases; walls curved.

Stomata on under surface, with thick lower walls; level with epidermis.

Palisade parenchyma two layers; broad, large, wedge-shaped cells; occupies 3/8 parenchyma space.

Spongy parenchyma twice as much as palisade, prominent air space; occupies 5/8 parenchyma space.

Summary: The slender leaves, the reflexed edges, and the thick cuticular wall indicate protective, water-retentive characters, but the abundant photosynthetic tissue with much air space would indicate sufficient available water.

Labiatae

Physostegia virginiana (L.) Benth.

Habitat: Alluvial basin; wet soil.

Orientation and arrangement: Horizontal, opposite; sessile.

Gross structure: Lanceolate to oblong; glabrous.

Histology: Bifacial (fig. 13, plate XI).

Epidermal cells moderate-sized; outer walls both about two times as thick as inner walls.

Stomata small; on the level with lower surface of epidermis.

Palisade parenchyma, cells broad; two layers, occupying about 1/2 parenchyma space.

Spongy parenchyma with large cells; much air space.

Summary: The surface stomata, the relatively thin-walled epidermis, the large-celled loose structure indicate photosynthetic activity and abundance of water without much tendency toward its conservation.

Mentha arvensis var. *canadensis* (L.) Briquet.

Habitat: Alluvial basin; damp soil.

Orientation and arrangement: Horizontal; opposite, lower leaves petioled.

Gross structure: Oblong to ovate; minutely pubescent.

Histology: Subcentric (fig. 12, plate XI).

Epidermis, both sides about equal in size, small; outer wall of upper 3 times as thick as inner wall; lower with walls of equal thickness. Upper wall with trichomes.

Stomata slightly depressed with interior cavity prominent.

Palisade parenchyma consisting of three upper and two lower layers; slender cells; occupies 3/4 parenchyma space.

Spongy parenchyma of two layers, resembling the palisade but twice as broad. Little air space.

Summary: The protective characters here seem to be the trichomes and the position of the stomata. Photosynthetic activity would seem to be prominent, according to the space provided for it.

Lycopus virginicus L.

Habitat: Alluvial basin; moist soil.

Orientation and arrangement: Ascending: petioled; opposite.

Gross structure: Ovate to ovate-oblong; puberulent.

Histology: Bifacial (figs. 14*a*, 14*b*, plate XI).

Upper epidermis thick-walled on outer side; lower epidermis uniformly thin-walled; wall of outer epidermis from a dry habitat twice as thick as that of a leaf from a moist habitat.

Palisade parenchyma 1 layer in leaves from a moist habitat; 3 layers in leaves from a dry habitat

Spongy parenchyma loose; occupies about the same space in each case, but equivalent to 2/3 the parenchyma space in the leaf from moist habitat and to 2/5 the parenchyma space in the leaf from dry habitat.

Stomata depressed in leaf of dry habitat; on level of epidermal cells in the other case.

Summary: The leaf from the dry habitat shows a thicker epidermal wall, depressed stomata and greater palisade tissues, indicating better conservational tendencies.

Scrophulariaceae

Mimulus ringens L.

Habitat: Alluvial basin; wet soil; near ponds.

Orientation and arrangement: Horizontal to ascending; sessile; clasping; opposite.

Gross structure: Oblong to lanceolate; glabrous.

Histology: Bifacial (figs. 15*a*, 15*b*, plate XI).

Variable epidermis; large-celled; fig. 15*a*, walls uniformly thin; fig. 15*b*, walls thickened on the outside.

Palisade parenchyma in fig. 15*a*, one layer; in fig. 15*b*, two layers.

Spongy parenchyma occupies about 1/2 parenchyma space in both figs. 15*a* and 15*b*, though the cells are nearly twice as long in fig. 15*a* as in fig. 15*b*.

Stomata near surface level.

Summary: Leaf shown in fig. 15*a* has no conservational devices; leaf in fig. 15*b* has evidently developed in this direction as shown by the thickened wall of the epidermis.

Compositae

Vernonia noveboracensis Willd.

Habitat: Alluvial basin; moist soil.

Orientation and arrangement: Horizontal to ascending; alternate; short-petioled.

Gross structure: Long lanceolate to lance-oblong; more or less pubescent beneath.

Histology: Subcentric (fig. 27, plate XIV).

Epidermis of uniformly thin-walled cells; mostly oblong, slightly hairy on upper surface; longer hairs on lower surface; an occasional glandular trichome on upper surface; epidermis dips down into the palisade in folds at intervals.

Palisade parenchyma loose; large-celled.

Vascular bundle surrounded by large cells, apparently water reservoirs.

Summary: Photosynthetic tissue prominent; the thin-walled epidermis contrasts with the fairly numerous trichomes on both surfaces and with the presence of water reservoirs.

Artemisia ludoviciana Nutt.

Habitat: Dry slopes.

Orientation and arrangement: Horizontal to ascending; alternate.

Gross structure: Lanceolate; upper mostly entire; lower cut-lobed, toothed, or pinnatifid; whitened woolly.

Histology: Bifacial (fig. 24, plate XIII).

Epidermal cells variable in size; thickness of walls variable, not much thicker on the outside than on the inside; trichomes numerous on both sides, but most on the upper side of leaf.

Stomata small, not much depressed.

Palisade parenchyma 2 layers, occupying 1/3 of the parenchyma space; large-celled.

Spongy parenchyma, cells large; rather loose.

Summary: Epidermal tissue not well developed except with regard to trichomes; photosynthetic tissue prominent.

Aster salicifolius Ait.

Habitat: Alluvial basin; low land; moist soil.

Orientation and arrangement: Horizontal to ascending; opposite; sessile.

Gross structure: Linear to linear-oblong; glabrous, sometimes scabrous.

Histology: Centric (fig. 17, plate XII).

Outer walls of epidermis about 3 times as thick as inside walls.

Stomata on both sides.

Palisade parenchyma, 2 layers on upper, 1 layer on lower side; occupies about 2/3 the parenchyma space. Cells medium-sized.

Spongy parenchyma, cells large; fairly compact.

Summary: Epidermis with fairly well developed cuticle though with stomata on both sides; photosynthetic tissue prominent.

Coreopsis palmata Nutt.

Habitat: Prairie slopes; dry to moist.

Orientation and arrangement: Ascending; sessile; opposite.

Gross structure: Wedge shaped; lobes broadly linear; glabrous.

Histology: Centric (fig. 20, plate XII).

Epidermis of small oblong cells; the outer walls thickened, 2 to 3 times the thickness of inner ones.

Palisade parenchyma occupies 2/3 of parenchyma space; 3 layers above, 2 layers below.

Spongy parenchyma, cells large but fairly compact.

Resin ducts present.

Summary: Outer wall of epidermis fairly well developed; photosynthetic tissue prominent.

Helianthus tuberosus L.

Habitat: Slopes and level; dry to moist soil.

Orientation and arrangement: Horizontal; alternate.

Gross structure: Oblong-lanceolate; scabrous above, pubescent below.

Histology: Bifacial (fig. 18, plate XII).

Upper epidermis with outer wall twice as thick as lower; wall of lower epidermis uniformly thin, but with numerous trichomes.

Palisade parenchyma of 3 layers, occupying 1/2 the parenchyma space.

Spongy parenchyma loose.

Summary: Photosynthetic tissue prominent; prominent air space; moderate conservational tendencies.

Helianthus grosseserratus Martens.

Habitat: Dry prairie; roadsides.

Orientation and arrangement: Horizontal to ascending; petioled; alternate.

Gross structure: Elongated lanceolate to ovate-lanceolate; glabrous above; finely pubescent beneath.

Histology: Bifacial (fig. 19, plate XII).

Outer wall of upper epidermis about 3 times as thick as inner; outer wall of lower epidermis about twice as thick as inner; cells small.

Palisade, 5 layers; medium-sized, occupying 2/3 of parenchyma space.

Spongy parenchyma loose.

Summary: Epidermal tissue better developed in *H. grosseserratus* than in *H. tuberosus.* Photosynthetic tissue is more compact.

Silphium laciniatum L.

Habitat: Rather dry priarie; sometimes moist slopes.

Orientation and arrangement: Stem leaves ascending; lower and root leaves vertical; alternate; petioled.

Gross structure: Pinnately parted, lobes lanceolate or linear, cut-lobed or pinnatifid; rough-bristly.

Histology: Subcentric (young leaf) (fig. 22, plate XII).

Epidermal cells square; with outer wall of epidermis 4 to 5 times as thick as inner wall.

Palisade in 5 layers, the longest at the top; fairly compact.

Summary: The well cuticularized epidermis indicates the ability to modify transpiration, while the prominent palisade implies pronounced photosynthetic activity.

Solidago rigida L.

Habitat: Dry soil; ridges and hillsides.

Orientation and arrangement: Ascending, somewhat appressed; petioled; upper leaves sessile.

Gross structure: Oval or oblong; rough-hoary with minute pubescence.

Histology: Concentric (fig. 26, plate XIV).

Epidermal cells small with outer walls 3 times as thick as inner; trichomes on both sides.

Stomata on both sides.

Palisade parenchyma, 3 layers on upper and 2 on lower side; loose; occupies 2/3 the parenchyma space.

Spongy parenchyma, small-celled; loose.

Stomata small, level with lower edge of epidermis.

Summary: Epidermis well equipped for conservation of water with the exception of stomata. Solereder states that stomata are often found on the upper sides of appressed leaves from dry habitats. Photosynthetic tissue well developed with prominent air space.

Solidago serotina Ait.

Habitat: Moist soil; slopes.

Orientation and arrangement: Horizontal to ascending, somewhat appressed; sessile.

Gross structure: Lanceolate to oblanceolate; glaucous.

Histology: Bifacial (fig. 23, plate XIII).

Epidermis of irregular-sized cells, convex-walled; outer wall about 1 1/2 times as thick as inner.

Stomata on level with lower edge of the epidermis.

Palisade parenchyma, 2 layers; broad-celled.

Spongy parenchyma, large-celled; rather loose; occupies 2/3 of parenchyma space.

Resin ducts present.

Summary: Has facilities for marked photosynthetic activity with slight protective device.

Solidago canadensis L.

Habitat: Moist soil; slopes.

Orientation and arrangement: Ascending, somewhat appressed; sessile.

Gross structure: Leaves narrowly lanceolate; glabrous above; minutely pubescent below.

Histology: Centric (fig. 25, plate XIII).

Epidermis thicker on upper than on lower side; outer walls about twice as thick as inner; trichomes on lower epidermis.

Stomata on both sides.

Palisade, 2 layers on upper surface and 1–2 on lower surface, occupying 2/3 the parenchyma space.

Spongy parenchyma of elongated cells running at right angles to the palisade; compact.

Resin ducts present.

Summary: A typical sun leaf with moderately developed protective devices to provide for active photosynthesis. Consistent with its moderately moist habitat.

Solidago graminifolia (L.) Salisb.

Habitat: Alluvial basin; moist soil.

Orientation and arrangement: Horizontal to ascending; sessile.

Gross structure: Lance-linear; glabrous.

Histology: Centric (fig. 21, plate XII).

Epidermal cells small; outer wall 1/3 thicker on upper than on lower side; outer wall of upper epidermis 3 times as thick as inner.

Palisade parenchyma, one layer on each side; cells rather small, oval; occupies 1/2 parenchyma space of leaf.

Spongy parenchyma cells large; fairly compact.

Summary: Leaf fairly thin without trichomes or prominent protective device; well developed palisade and spongy parenchyma.

Comparison of Leaf Anatomy of Upland and Alluvial Basin Plants

The following plants were selected from two formations:

I. Prairie hill crest and slope: *Andropogon scoparius, Bouteloua curtipendula, Anemone cylindrica, Amorpha canescens, Ceanothus americanus, Eryngium yuccaefolium, Artemisia canadensis, Solidago canadensis, Solidago rigida, Coreopsis palmata, Helianthus grosseserratus, Helianthus tuberosus.*

Epidermis. Of these twelve upland plants, all have a relatively thick outer-walled epidermis, or prominent trichomes if the wall is not thick. The presence of trichomes may be regarded as an anatomical equivalent of thick-walled epidermis. Two have prominent bulliform cells and vascular bundles. (Not enough data are recorded for stomata to warrant comparisons.)

Parenchyma:

- Subcentric leaves.
  - (1) Five layers palisade parenchyma.
  - (2) Four layers palisade.
- Centric.
  - (1) Three upper, two lower layers palisade; spongy parenchyma loose.
  - (2) Three upper, two lower layers palisade; spongy parenchyma large-celled, compact.
  - (3) One upper, one lower layer palisade; spongy parenchyma large-celled, compact.
  - (4) One upper, one lower layer palisade; spongy parenchyma horizontally elongated.
- Bifacial.
  - (1) Two layers of palisade cells; spongy parenchyma cells compact, small.
  - (2) Two layers of palisade cells; spongy parenchyma cells large, compact.
  - (3) Three layers of palisade cells; spongy parenchyma cells loose, elongated.
  - (4) Four layers of palisade cells; spongy parenchyma cells loose, elongated.
- Radial palisade.
  - (1) Radial palisade; spongy parenchyma homogeneous.
  - (2) Radial palisade; spongy parenchyma homogeneous.

II. Alluvial basin: *Leersia oryzoides, Muhlenbergia mexicana, Polygonum Muhlenbergii, Baptisia leucantha, Steironema lanceolata, Asclepias verticillata, Apocynum cannabinum, Mimulus ringens, Lycopus virginicus, Mentha canadensis, Physostegia virginiana, Solidago serotina, Solidago graminifolia, Vernonia noveboracensis, Silphium laciniatum, Aster salicifolius.*

Epidermis: Of the sixteen alluvial basin plants examined, nine have a lower epidermal wall relatively thinner than that on the upper side. Ten are smooth.

Parenchyma:

- Subcentric.
  - (1) Five layers palisade.
  - (2) Six layers palisade.
  - (3) Seven layers palisade.
- Centric.
  - (1) One upper, two lower layers palisade; spongy parenchyma compact.
  - (2) Two upper, two lower layers palisade; spongy parenchyma compact.

Bifacial.
- (1) One layer palisade; spongy parenchyma loose.
- (2) One layer palisade; spongy parenchyma loose.
- (3) Two layers palisade; spongy parenchyma loose.
- (4) Two layers palisade; spongy parenchyma loose.
- (5) One-two layers palisade; spongy parenchyma loose.
- (6) Three layers palisade; spongy parenchyma loose.
- (7) One-three layers palisade; spongy parenchyma loose.

Homogeneous.
- (1) Rather compact mesophyll (Grass).
- (2) Rather compact mesophyll (Grass).

Mimulus ringens studied in two habitats shows in one location two layers of palisade and a relatively thick outer epidermis, while in the moister habitat it has a uniformly thin epidermis and one layer of palisade with a thicker area of spongy parenchyma.

Polygonum Muhlenbergii shows a development of trichomes and a thicker palisade in leaves growing a few rods from a pond, while in shallow water its leaves are smooth and have a narrower palisade.

The Compositae and Gramineae by far exceed the other families in both number of representative species and individuals thereof.

Compositae. The Compositae show considerable variation of tissues, but are characterized by prominent palisade, four of the leaves having a centric structure, two subcentric, and four bifacial with deep palisade. The spongy tissue where present is loose. On low, wet land trichomes are generally not developed. Thickened epidermal walls are representative. The leaves are characterized by intensive photosynthetic activities. A tissue may be represented by an anatomical equivalent under different conditions indicating adaptability to habitat, manifested not only in anatomical characters but through evidence of wide distribution and great numbers.

All these Solidagos have well developed palisade but each of a different type. *S. rigida* has the driest habitat and shows the most prominent palisade, cuticle, and trichome development. The leaves appressed to the stem are associated with the presence of stomata on both sides. *S. serotina* has a moist habitat and shows practically no protective devices, having epidermal walls with loose spongy parenchyma. *S. graminifolia,* though growing in as moist, if not a moister, habitat than *S. serotina,* has a thicker cuticle and more compact parenchyma with two layers of palisade. *S. canadensis* has a fairly dry habitat though not so dry as that of *S. rigida.* Its compact tissue, prominent palisade, and thick-walled hairy epidermis seem consistent with its location.

Gramineae. The grasses of the lowland show a thinner epidermis, fewer and smaller bulliform cells, and less specialized palisade in the alluvial basin than in the upland prairie. Among the contributions dealing with Iowa grasses are the following: Emma Pammel Hansen (12) studied the anatomy of *Lolium perenne*, *Festuca elatior*, *F. tenella*, and *Bromus patulus*. Emma Sirrine (17) described and illustrated the anatomy of *Bromus patulus*, *B. inermis*, and *B. secalinus*. Pammel and Sirrine (13) investigated the anatomy of *Sporobolus heterolepis*, *S. cryptandrus*, *S. Hookeri*, *S. vaginaeflorus*, *Panicum capillare*, *P. proliferum*, and *P. crusgalli*. These investigators call attention to the difference in anatomy of plants growing in dry and in humid environments. C. R. Ball (2) examined *Eragrostis reptans*, *E. pectinacea*, *E. Purshii*, *E. Frankii*, *E. Mexicana*, and *E. major*, and describes the epidermal cells of *E. pectinacea* as having thicker walls than those of *E. Purshii*, the latter being adapted to dry and sandy soil. C. B. Weaver (21) worked upon the anatomy of *Andropogon nutans*, *A. scoparius*, *A. sorghum*, and *A. sorghum* var. *halepense*. Miss Pammel's studies of Bromus show little development of bulliform cells and a homogeneous mesophyll, in contrast with the prominent bulliform cells of reduced spongy parenchyma and the radial palisade of Weaver's Andropogon and Ball's Eragrostis, both species of dry habitats. Theo. Holm (8) has studied the species of the genera Uniola, Distichlis, Pleuropogon and Leersia. Pammel, Weems, and Lamson-Scribner (11) have called attention not only to the use of anatomical characters of grasses for systematic distinction but to such structures as related to habitat. An interpretation of the above cited morphological facts concerning a number of species of a genus and a comparison of genera with a knowledge of the habitats of these plants would leave no doubt as to the adaptation of species of this family to their habitats.

In general, the anatomy of these leaves of prairie plants resembles that of the plants described in Harshberger's (7) pine barren studies in their development of epidermis and mesophyll features.

Ella Shimek (16) has described plants of Iowa prairies with some illustrations of leaf characters, in which the leaf structure coincides with the anatomical observations of the present study.

SUMMARY

The leaves of prairie plants show a xerophytic tendency in their leaf structure, indicated by the specialized palisade tissue, the thick-walled and trichomeless epidermis, the presence of water-storing tissue, and sometimes of trichomes.

The mere presence of these characters is not of primary significance as an indication of xerophytism, but their relative development correlated with other morphological features of the plant such as the extensiveness of the root system.

The upland plants have a thinner epidermis than those of the lowland, and 70 percent of those studied are without trichomes while 75 percent of the upland species have trichomes.

Of the alluvial basin leaves studied, 50 percent were bifacial while 33 percent of the upland plants have bifacial leaves; 12½ percent of the alluvial basin plants were centric to subcentric; 50 percent of the upland plants were centric to subcentric.

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EXPLANATION OF PLATES IX-XIV

The figures were made with the aid of a camera lucida and are magnified approximately 30 diameters.

Ed, dorsal epidermis; *Ev*, ventral epidermis; *P*, palisade parenchyma; *Sp*, spongy parenchyma; *VB*, vascular bundle; *b*, bulliform cell; *r*, resin duct; *s*, stomate; *t*, trichome; *cps*, chlorophyll parenchyma sheath.

PLATE IX. Fig. 1, *Andropogon scoparius;* Fig. 2, *Bouteloua curtipendula;* Fig. 3, *Muhlenbergia mexicana;* Fig. 4, *Leersia oryzoides;* Fig. 5, *a* and *b*, *Polygonum Muhlenbergii;* Fig. 6, *Anemone cylindrica.*

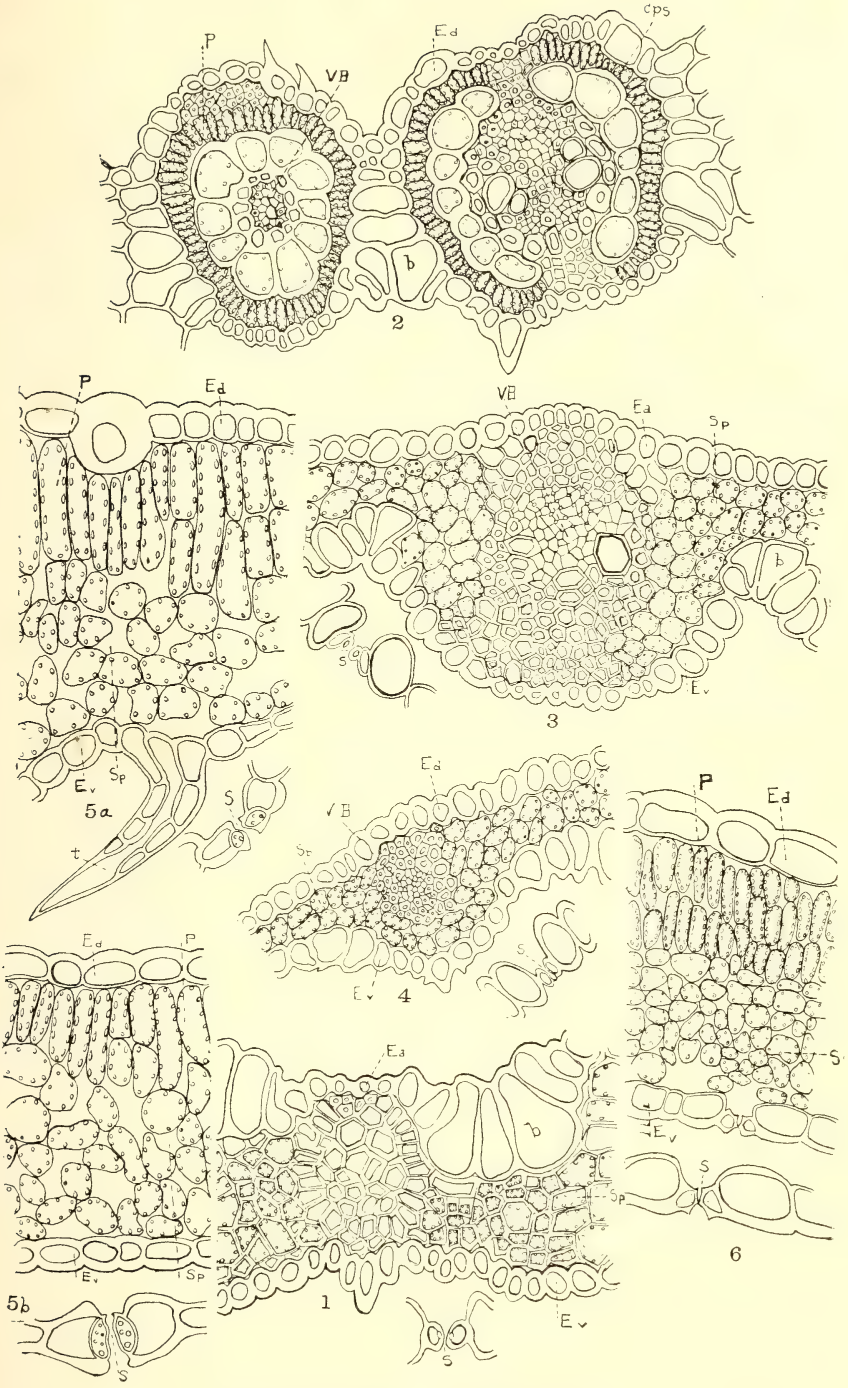
PLATE X. Fig. 6*a*, *Baptisia leucantha;* Fig. 7, *Amorpha canescens;* Fig. 8, *Ceanothus americanus;* Fig. 9, *Steironema lanceolata;* Fig. 10, *Apocynum cannabinum;* Fig. 11, *Asclepias verticillata.*

PLATE XI. FIG. 12, *Mentha arvensis* var. *canadensis;* Fig. 13, *Physostegia virginiana;* Fig. 14, *a* and *b*, *Lycopus virginicus;* Fig. 15, *a* and *b*, *Mimulus ringens;* Fig. 16, *Eryngium yuccaefolium.*

PLATE XII. FIG. 17, *Aster salicifolius;* Fig. 18, *Helianthus tuberosus;* Fig. 19, *Helianthus grosseserratus;* Fig. 20, *Coreopsis palmata;* Fig. 21, *Solidago graminifolia;* Fig. 22, *Silphium laciniatum.*

PLATE XIII. Fig. 23, *Solidago serotina;* Fig. 24, *Artemisia ludoviciana;* Fig. 25, *Solidago canadensis.*

PLATE XIV. Fig. 26, *Solidago rigida;* Fig. 27, *Vernonia noveboracensis.*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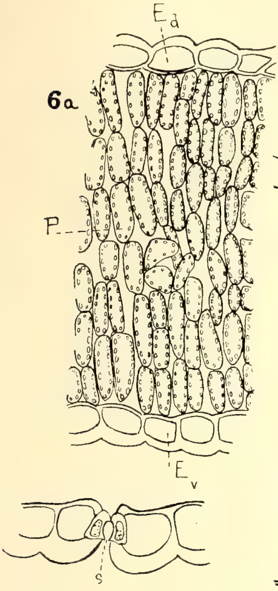

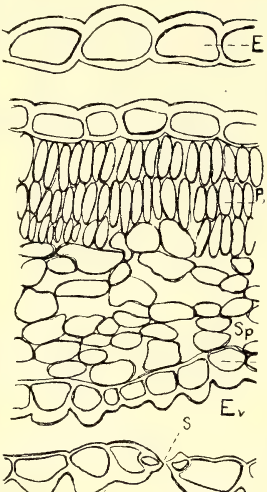

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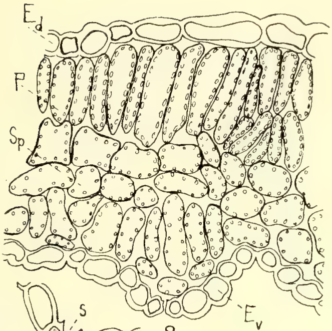

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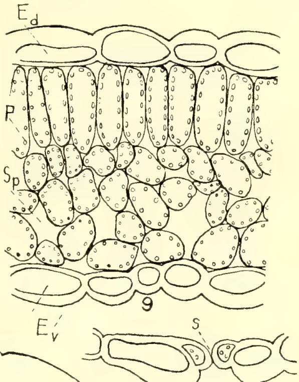

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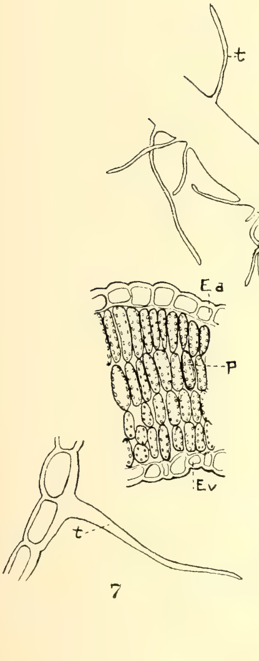

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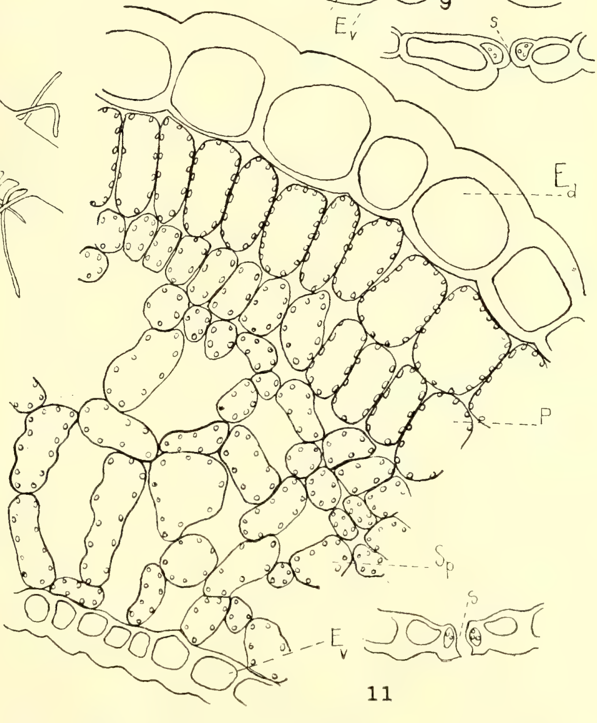

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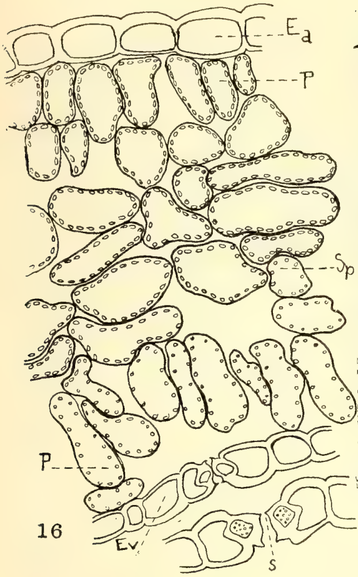

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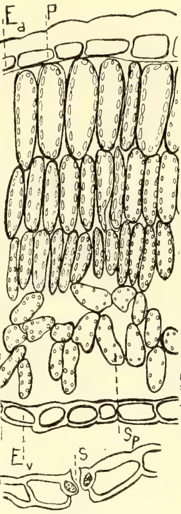

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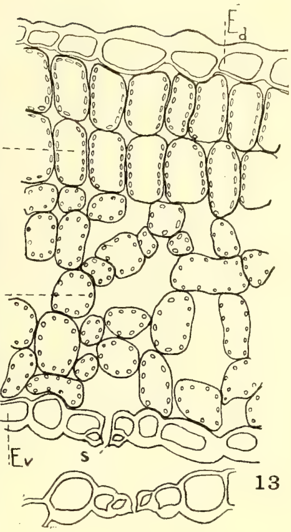

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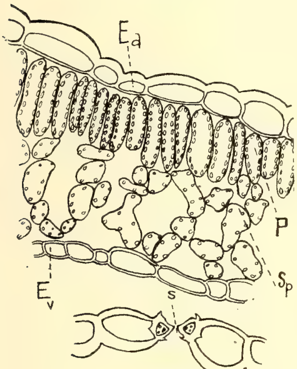

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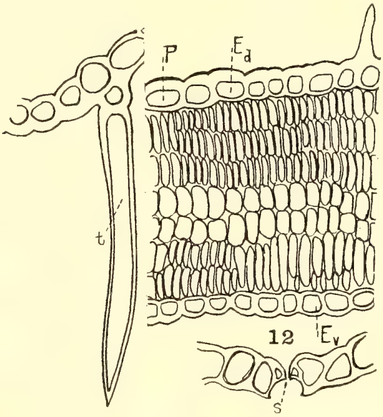

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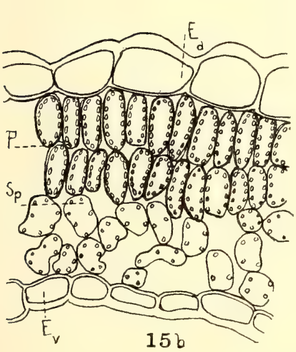

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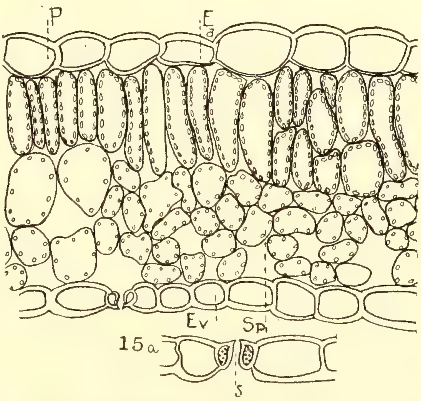

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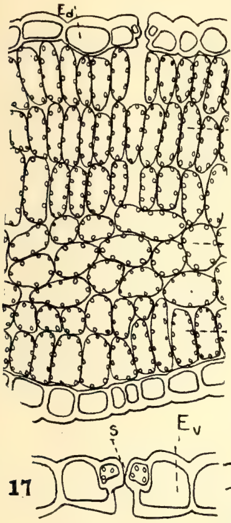

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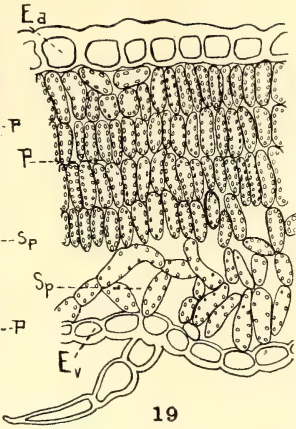

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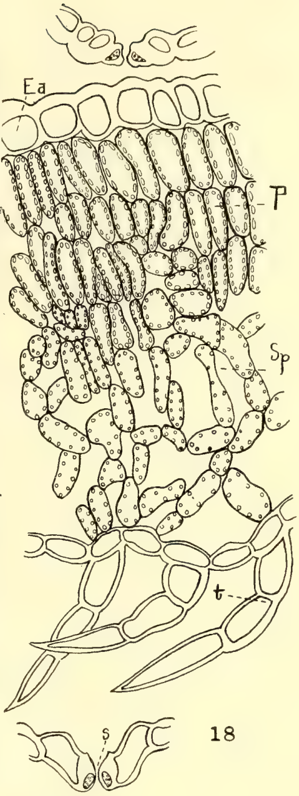

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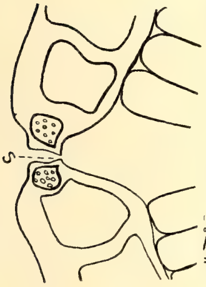

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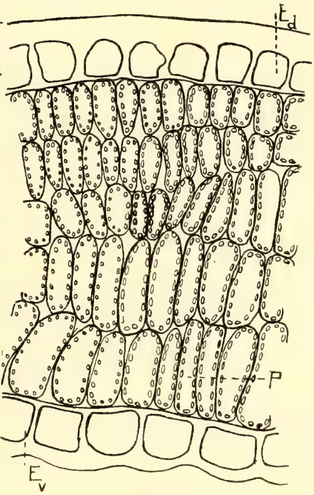

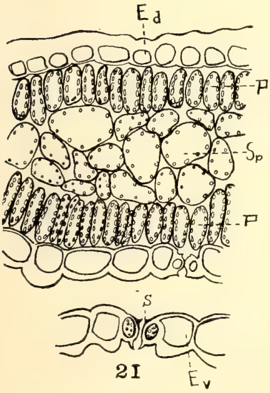

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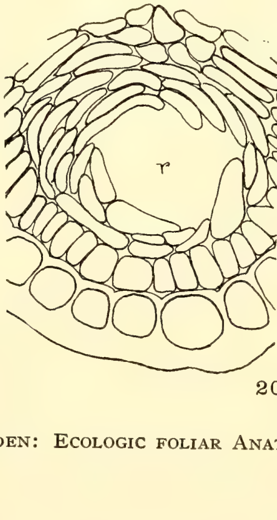

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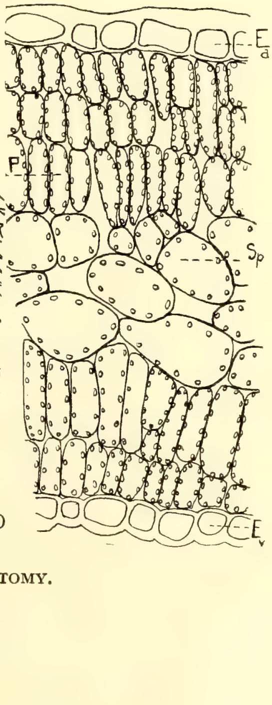

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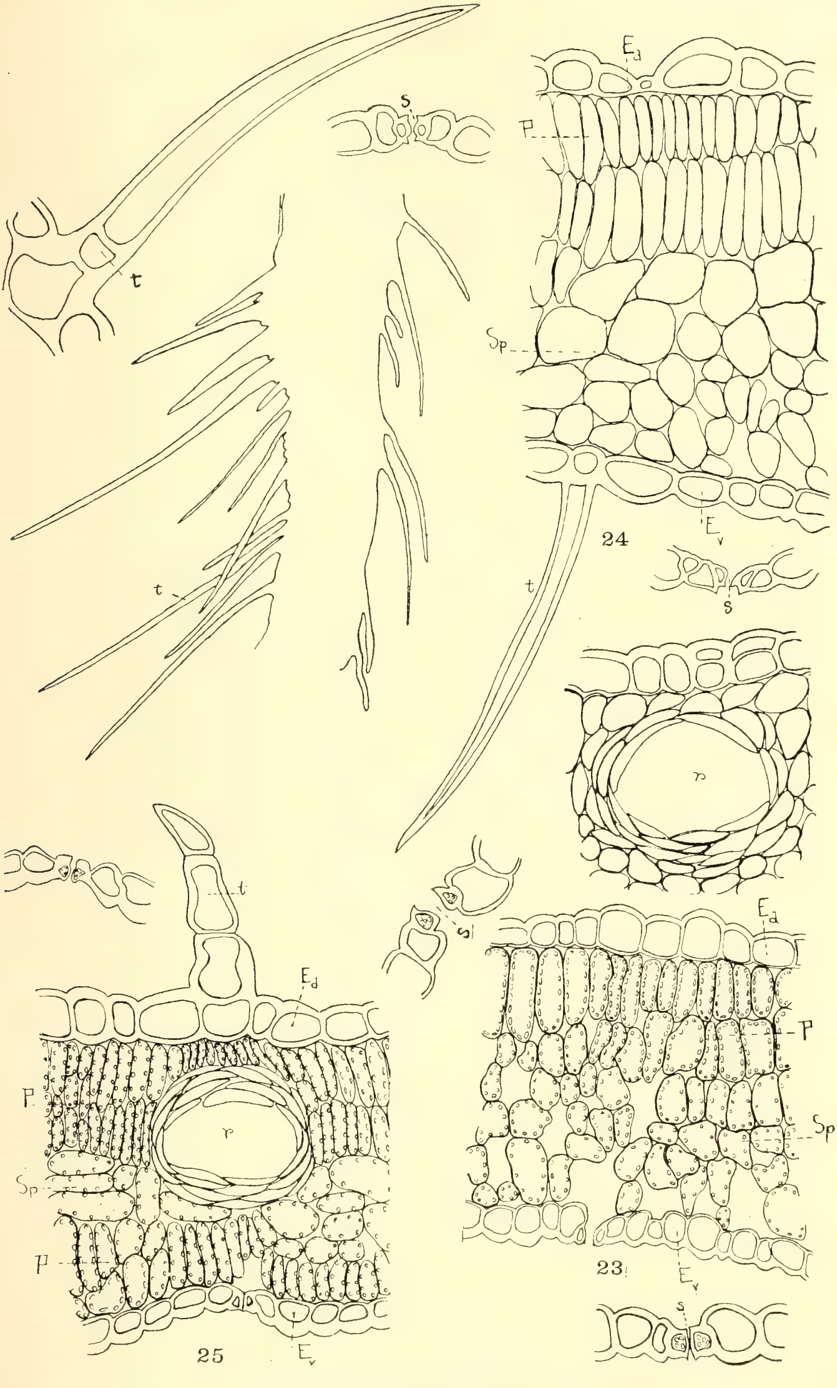
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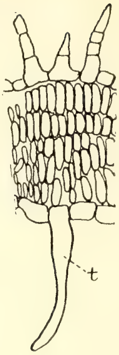

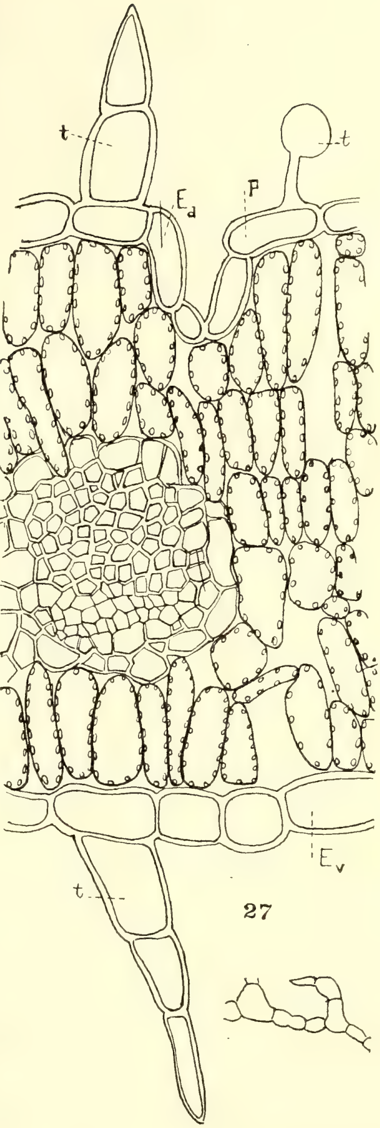

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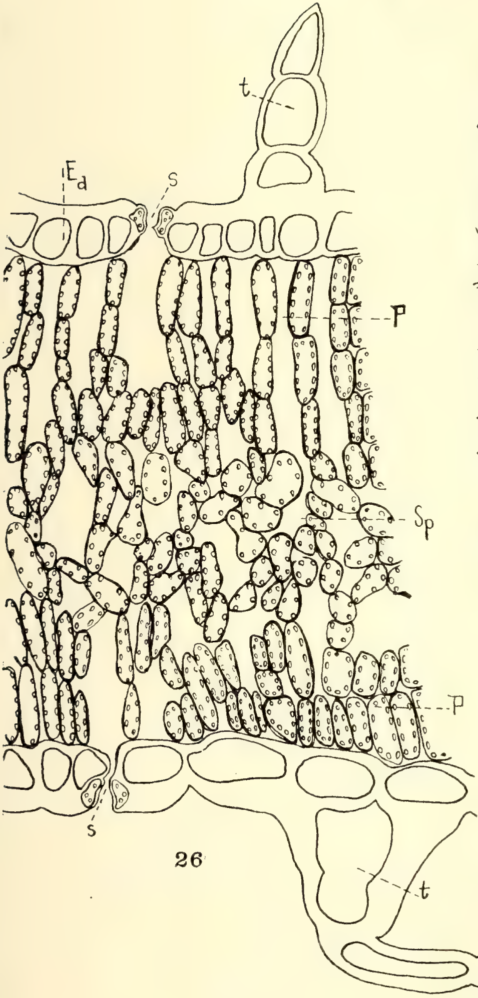
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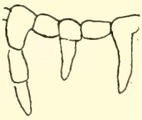

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PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT 41 NORTH QUEEN STREET, LANCASTER, PA.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3,1 879

AMERICAN JOURNAL OF BOTANY

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MAR 31 1919
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AMERICAN
JOURNAL OF BOTANY

VOL. VI MARCH, 1919 NO. 3

THE ECOLOGIC SUBTERRANEAN ANATOMY OF SOME PLANTS OF A PRAIRIE PROVINCE IN CENTRAL IOWA

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INTRODUCTION

A survey of the literature pertaining to functional and adaptational features of subterranean organs as related to their morphology shows this subject to have been less investigated than that of leaf structure. Though there has been considerable work done in the field of pure morphology, that too is yet deficient in many respects. Descriptions found in Gray's Manual of Botany (8) and Britton and Brown's Flora (1) have either no reference to subterranean parts or only incomplete ones.

The subterranean organs of prairie plants are of two classes, roots and subterranean stems. Of course all these plants have secondary, *i. e.*, absorbent or collecting roots, but in many plants the subterranean stems are intermediate structures connecting collecting roots with conducting aërial stems and therefore assume the function of primary or main roots.

Prominent subterranean stems are seen in the majority of alluvial basin plants and in modified abbreviated form in the upland plants, especially in the grasses and composites which constitute the majority of the upland plants.

It was the object of this study of subterranean stem and root types (*a*) to note whether variations were correlated with habitat; (*b*) to determine whether the stems were equivalent to or advantageous to the root in the economy of the plant.

The *functions* of subterranean organs are *storage*, *anchorage*, *absorption*, *conduction*, and *propagation*. Anchorage in the case of prairie plants may be dismissed, for most of them are low, have a compact habit of growth, and are not particularly subject to uprooting. As to *propagation*, the fact that subterranean stems have numerous nodal buds while roots have adventitious buds, if any, gives subterranean stems, especially rhizomes, the advantage as propagators. Concerning *storage*, the root and the subterranean stem seem to be equally well equipped, though this question will be discussed under anatomical structure.

[The **Journal** for February (**6**: 47–86) was issued March 1, 1919.]

METHODS

The material was stored in 50% alcohol, and after free-hand cutting the sections were stained with a solution of water-soluble safranin, followed by haematoxylin, cleared in cedar oil and clove oil, and mounted in Canada balsam. The drawings of the subterranean structures were not made to a single scale because the large size of some of them made this impracticable, and also because of the variability in size, which makes the comparison of the size of the roots and stems of species less important than the relative proportion occupied by the tissues of the roots compared.

HISTORICAL

Some progress has recently been made in classifying subterranean organs; among the valuable contributions are those of Cannon (2), Harshberger (11), Holm (13), Yapp (18), Dauphiné (6), Constantin, Jodin (15), and Maxwell (16).

Maxwell (16) reviews the history of the histological study of roots, which before 1865 were studied as masses of tissues and after this period with reference more directly to the origin of organs.

According to Cannon (2), the work of Rimbach, Büsgin, and Friedenfeldt as reviewed by Von Alter (Wurzelstudien, Bot. Zeit. **67**: 175. 1909) is important because their researches indicate that the root systems of flowering plants may be divided into two groups according to the character of the terminal roots; they are either *intensive* or *extensive*. Intensive root systems have fine terminal roots; they are richly branched and occupy small soil volume. Extensive root systems have coarse ultimate roots, are not richly branched, and occupy a relatively large soil volume. Cannon describes three main types of root systems found in the desert plants of the southwestern United States: (*a*) Root systems which extend horizontally from the main axis and lie for their whole course near the surface of the ground. (*b*) Root systems which are characterized by a strongly developed tap root going down directly to a depth determined in part by the character of the soil, in part by the penetration of the rains, and in part by the character of the root itself. (*c*) Roots that not only reach widely but penetrate fairly deeply. When the root is of an obligate type the distribution of the species is much restricted, but when it undergoes modification with changed environment the distribution of the species is much less confined.

Yapp (18) and Scherff (17), in their marsh studies, note the stratification of subterranean systems as well as the aërial portions of different species.

Holm (13) reviews a paper of Häckel (10) on the peculiarities of the grasses of dry climates, among which he distinguishes (*a*) *tuberous* and *bulbous grasses* and (*b*) *tunic grasses.* The tuberous and bulbous forms occur only in countries with periodic dry seasons. None have been observed in the moist parts of the tropical region. The author does not regard these bulbs or tubers as reservoirs of starch or sugar, as is true of the similar organs of the Liliaceae and Iridaceae. Though they are structurally homologous with these, physiologically they are reservoirs. The author has shown that *Poa bulbosa* on being cultivated in moist soil almost loses its bulbous character. The second group includes forms in which the bases of the culms and shoots are covered with at least three faded sheaths. These all inhabit dry localities. In those forms which prefer damp or shaded places there is usually one faded sheath present, and even that disappears soon. *Straw tunics* are distinguished from *fiber tunics*; in the former the sheath remains complete although faded, in the latter the sheath breaks up into fibers. The function of these tunics is regarded as that of water conservation. Holm has contributed various studies on the morphology of subterranean portions of plants.

Gross Anatomy

In the alluvial basin region the prairie plants under observation show a prominent development of thickened, elongated rootstocks. The majority of these plants are Gramineae, Cyperaceae, and Compositae, in which this feature is a systematic character. The Cyperaceae are seldom found on the highland, but the upland members of the Compositae and Gramineae, while they retain their rootstock characters, have abbreviated forms. The Gramineae especially show abbreviated subterranean parts in the form of hard bulbous or corm-like thickenings from which the roots radiate, or of short hard rootstocks. *Panicum virgatum*, which has a wide range of habitat, growing either on upland or lowland, has hard, radially branching, slender, scaly rootstocks which are shorter in the drier upland habitats. *Spartina Michauxii*, which grows on lowland or in moist upland ravines subject to drought, shows shorter rhizomes on the upland. The Solidagos which frequent the ridges have very abbreviated, hard, corm-like subterranean stems, while those which frequent moist habitats have longer rhizome-like structures. The genus Liatris has a species with short, sheathed corm which grows on the hilltops and a species with an elongated, fibrous-sheathed tuber on the moister slopes. *Helianthus grosseserratus* has a short, thick rootstock, while *H. tuberosus* has a long, slender-stemmed tuber. *Silphium laciniatum* is provided with a deep root. The Compositae are variable, having short tubers, elongated rhizomes, corm-like structures, or tap and fascicled roots, which enable them to thrive in a wide range of habitats and probably account for their great numbers and diversity of forms. Among the Leguminosae are *Desmodium illinoense*, *Amorpha canescens*, *Lespedeza canadensis*, *Petalostemum purpureum*, and *P. albidum*, which are upland plants with deep, tough roots, usually having long, thick tap roots and somewhat smaller lateral roots. These leguminous plants and others with similar root habits show little storage area but have prominent tracheae, which would indicate that they derive their moisture from a lower water table than do their short-rooted neighbors. Liatris shows few tracheae but much storage tissue. Ceanothus has also a deep, branched tap root. The exterior of the cortex of the roots is corky, leathery, fibrous-sheathed, or flaky-deciduous, while the subterranean stems have hard, scaly fibrous or straw sheaths which probably have the ability to hold water by capillarity as well as to prevent evaporation from the inner parts. These scaly coverings of thickened subterranean organs, especially of rhizomes, are prominent in both uplands and alluvial basins. Since conditions of drought are likely to occur frequently and especially in protracted periods during the latter part of the summer, these features are undoubtedly useful as a protection against desiccation. The swamp plants must be able to tolerate not only moisture to the point of saturation but drought as well. The roots of the upland plants may be regarded as of the intensive type, those of the lowlands as of the extensive type.

Minute Anatomy

The anatomical descriptions of the stems and roots studied have been made under the heads (*a*) Primary cortex, and (*b*) Stele, which portions of the stem and root are homologous. These structures show certain prevalent types which may be distinguished in the angiosperms as follows:

Monocotyledons—
    Stem: concentric or collateral, endarch bundles.
    Root: radial, exarch bundles.
Dicotyledons—
    Stem: concentric, endarch bundles.
    Root: radial, exarch bundles.

There is great diversity of structure shown by the representatives of different families and individuals thereof, whose morphological and physiological variations are discussed by De Bary, Solereder, Haberlandt, Stevens, Coulter, Barnes, Cowles, and others. Exceptions seem more abundant than cases of conformity to rules at the present status of correlation. Transformations from the original types take place with the secondary thickening process, in numerous instances to such an extent that the original type structures are hardly recognizable. Systematic relations have not been conclusively worked out, yet every species has in some degree established its economy of water relations, the indicators of which, in so far as experiment has proceeded, are shown to be primarily (*a*) *parenchyma* (storage and aerenchyma), (*b*) *mechanical tissue*, and (*c*) *conductive tissue*. The prominence of the latter two seems to indicate xerophytism and that of the first the reverse condition. *The relative proportion of these tissues in each individual stem or root has been used as a means of indicating its degree of xerophytism* in this study.

Before applying these tests perhaps the reasons for selecting them should be given. An examination of young water-absorbing roots shows them to have a *deep cortex bearing root hairs and a small vascular cylinder with distinctly radial bundles.* "In roots, any departures from the typical radial structure of the vascular strands are generally correlated with special environmental conditions, or arise from the necessity of increasing the amount of available conducting tissue" (Haberlandt). In a radial root there is no means of tangential increase, so this increase must take place in a radial direction toward the cortex and results in orienting a cambium which produces concentric layers of phloem and xylem. This is seen to be an advantageous structure in older roots whose function is conduction and not absorption from the cortical layer, for here there is no incoming stream of water to cross the proteid-conducting zone but only a rising central column. It is seen that such an arrangement is also desirable for resistance to strains which in roots are in a longitudinal direction. This solid cylinder gradually develops pith and assumes an annular vascular structure in the stem, from which bundles shoot out into the branches. Hence a root changes from a water-collecting to a water-radiating organ and the pith of the stem serves as a good collecting reservoir; though pith is sometimes absent. The stem, being subject to radial strains, is thus well adapted by its hollow-cylinder mechanical system.

Rhizomes (Haberlandt) *which fix the plant in the soil* agree with roots in having their mechanical tissues united to form a stout axile tube or a solid central strand; this centralization of the mechanical system is very marked in the rhizomes of grasses, sedges, and rushes, which, accordingly, when regarded from an anatomic-physiological standpoint, approximate more closely to roots than to the aerial stems of which they are the morphological equivalents.

Structural features (Solereder) *which vary with the amount of water in the soil* and air and with the degree of transpiration on the part of the plant, affecting chiefly the number of vessels and width of lumina, are of minor systematic value.

Kohl observed that certain plants (*Mentha aquatica*, *Thalictrum galeoides*, and *Menyanthes trifoliata*) develop more collenchyma and bast if grown in dry air, *i. e.*, under conditions favorable to transpiration, than they would if produced in a moist atmosphere, *i. e.*, with their transpiration reduced. Here it is impossible to state with certainty whether the process is adaptive or self-regulatory. It should, however, be noted that in the case of herbaceous plants growing in a dry atmosphere, or in fact under xerophytic conditions in general, turgor has a smaller mechanical value than usual because the risk of temporary wetting is so great in these circumstances that any decrease in the development of mechanical tissue must be advantageous. In general, there is correlation between the number of water-conducting vessels and the extent of the foliar transpiring surface.

Jost, by removing the leaves of seedlings of *Phaseolus multiflorus*, *Helianthus annuus*, and *Vicia Faba*, found that the vascular bundles supplying the amputated leaves remained rudimentary. There is evidence of a process of adaptive self-regulation.

Kohl has demonstrated that the water-conducting system may be reduced by growing the plants in a moist atmosphere, and Schenck has demonstrated the reduction of vascular bundles of *Cardamine pratensis* by growing the terrestrial plant in water. Plants which are naturally amphibious exhibit a similar character.

The endodermal layer has been a subject of considerable experiment and study. In some plants the cells of this layer have thickened inner walls, and in some the walls are of uniform thickness. Schwendener has shown that the endodermis is impermeable except in spots where it acts as side sluices in a system of irrigating canals, the main channels of which are represented by the vessels. Greatly thickened endodermal layers are found in marsh plants which live in places likely to dry up periodically. This is true of some plants growing in dry regions.

Gaseous exchange is slow under aquatic conditions and is accelerated by the presence of air spaces. Aerenchyma is also found in marsh plants subject to submerging.

These facts show that the relative distribution and proportion of mechanical tissue, parenchyma, and conductive cells are of considerable significance with reference to adaptation in the economy of plant tissues.

DESCRIPTION OF SUBTERRANEAN ORGANS

Typhaceae

Typha latifolia L.

Habitat: Alluvial basin; marsh.

Gross structure: Long, stout, horizontal rootstocks; 6–8 in. deep; soft and spongy, origin from a thick stem base; long, slender; straight roots, at notes, perennial (Plate XV, fig. *d*).

Histology of rhizome (Plate XVI, fig. 1):

Primary cortex: Radius of cortical area ½ radius of rhizome; hypodermis, thin-walled parenchyma; next a zone of rounded-compact parenchyma in which vascular bundles are originating; zone of aerenchyma in which vascular bundles have enlarged; bundles surrounded by woody sheath. Endodermis pronounced, with thick inner wall.

Stele: Composed of one row of large, woody bundles surrounding the pith cylinder of thin-walled aerenchyma, among which are scattered smaller vascular bundles with compact, thick, woody sheath.

Summary: Air space prominent; parenchyma well developed; vascular tissue fairly abundant; slight mechanical tissue, found only at sheath for bundles; vascular strands act as mechanical tissue in cortex.

Histology of root (Plate XVI, fig. 2):

Primary cortex: Composed of 4-sided to oval parenchyma cells; air space small; no mechanical tissue.

Stele: Radius 4/5 that of the root; conducting vessels fairly prominent, surrounded by parenchyma.

Gramineae

Phragmites communis Trin.

Habitat: Alluvial basin; edge of water, wet soil.

Gross structure: Long, creeping rhizome, thick, soft and spongy; scales at nodes prominent; clusters of roots emerging from nodes bearing fine secondary hair-like roots; rhizomes branch in several directions and at several levels; perennial (Plate XV, fig. *b*).

Histology of rhizome (Plate XVII, fig. 1):

Primary cortex: Radius equal to 1/2 radius of rhizome; epidermal cells small, thick-walled; hypodermis of thin collenchyma; parenchyma large-celled, hexangular, forming arches around large oval air cavities in a ring within cortical cylinder.

Stele: Hypodermis, 3 rows of collenchyma; main portion of vascular cylinder composed of large thin-walled parenchyma surrounding rows of alternately arranged vascular bundles; vascular bundles sheathed by wood fibers; one row of small bundles where arches of cortical parenchyma join vascular cylinder.

Summary: Rhizome characterized by prominent thin-walled cortical cylinder; mechanical tissue reduced; vascular bundles few.

Histology of root (Plate XVII, fig. 2):

Primary cortex; Radius 5/7 of root radius; hypodermis, 3 rows thin collenchyma; parenchyma of large oblong cells with long axes radially placed; large elliptical air spaces; endodermis prominent, with thick outer wall.

Stele: Composed of small thin-walled parenchyma cells with a ring of young radial vascular bundles in which the exarch arrangement of metaxylem and protoxylem is very distinct.

Summary: Young root composed almost entirely of thin-walled, loosely arranged parenchyma with prominent air space, only a trace of mechanical tissue.

Spartina Michauxiana Hitchc.

Habitat: Alluvial basin; wet ground; sometimes fairly dry locations.

Gross structure: Creeping radial rhizome; long, tough, hard; scaly; springs from a short, condensed base; perennial (Plate XV, fig. *h*):

Histology of rhizome (Plate XVIII, fig. 1):

Primary cortex: Radial depth 3/7 radius of rhizome; epidermis small-celled, thick, walled; hypodermis, 7 rows of collenchyma in which occasional small vascular bundles are embedded; clusters of compact, round parenchyma cells alternating with large air spaces constitute the inner arc of the cortex; endodermis thick-walled.

Stele: Sclerenchyma of 6 rows borders outer margin and hollow center of this cylinder; vascular bundles numerous, large, with prominent sheath of wood fibers; pith parenchyma relatively thick-walled; only a few cells of pith between bundles.

Summary: Parenchyma and air space reduced; mechanical tissue prominent; vascular bundles numerous, large.

Panicum virgatum L.

Habitat: Alluvial basin; low, wet soil; sometimes moist; dry upland.

Gross structure: Plant tufted, with long, creeping rhizomes; scaly, hard and tough perennial.

Histology of rhizome (Plate XVIII, fig. 2):

Primary cortex: Radius 2/9 that of rhizome; epidermal cells 4-sided, thick-walled, small; hypodermis, 7–8 rows of thick collenchyma; inner half of cortex composed of clusters of thin-walled, compact parenchyma alternating with large air spaces.

Stele: Vascular bundles large, numerous, surrounded by thick sheaths of sclerenchyma; pith parenchyma of 2–3 rows separating the bundles; bands of sclerenchyma 3–4 cells deep bound the perimeter of the vascular cylinder and surround the hollow center.

Summary: Parenchyma poorly developed; air space small; mechanical tissue prominent; vascular bundles large, strongly sheathed.

Cyperaceae

Scirpus fluviatilis (Torr.) Gray.

Habitat: Alluvial basin; swamps.

Gross structure: Rhizome elongated, terminating in tuber-like swellings; radial growth; moderately thick; hard tuberous tip but spongy root-stalk; horizontal, descending; perennial (Plate XV, fig. *c*).

Histology of rhizome (Plate XIX, fig. 3):

Primary cortex: Radius 1/2 that of the rhizome; thin-walled, angled to oval parenchyma cells form a band 1/5 the depth of the cortex and send down rays to the vascular cylinder, forming arches around the large air spaces; endodermis cells distinct, of uniform thickness.

Stele: Just inside the endodermis one row of concentric vascular bundles strongly sheathed with wood fibers; woody sheathed bundles scattered through the parenchyma; parenchyma compact; thin-walled.

Summary: Air cavities prominent in cortex; parenchyma thin-walled; vascular bundles numerous; parenchyma fairly prominent in central cylinder.

Scirpus validus Vahl.

Habitat: Alluvial basin; margin of pond.

Gross structure: Rhizome stout, scaly; horizontal; linear extension; cortex soft and absorbent; vascular cylinder hard and flinty; fringes of slender roots with secondary hair-like branches radiate from the short nodes, appearing continuous not clustered; perennial (Plate XV, fig. *g*).

Histology of rhizome:

Primary cortex: Radius 1/3 that of the rhizome; air space prominent; parenchyma cells round, with 5–6 spoke-like, short cellular flanges forming a characteristic network; endodermis prominent with thick inner walls.

Stele: Solid; pith parenchyma cells round to oval, rather thick-walled; concentric bundles numerous around the outer perimeter of cylinder, but larger and fewer toward center; bundles thickly sheathed by centripetal arc of bast.

Summary: Aerenchyma prominent; this type of cell structure not only provides ample air space but insures mechanical strength; vascular tissue prominent.

Iridaceae

Iris versicolor L.

Habitat: Alluvial basin; margin of pond, swamp.

Gross structure: Rhizome thick, relatively short; tuberous swellings; horizontal; linear extension; roots slender, vertically descending, clustered at nodes; fine secondary branches; perennial (Plate XV, fig. *e*).

Histology of rhizome (Plate XIX, fig. 1):

Primary cortex: Radius 1/4 that of rhizome; 3 rows hypodermal mechanical tissue; a similar band of angular cells with slightly thickened walls bordering the inner arc of cortex; aerenchyma a hexangular network; endodermis thick-walled on inner surface.

Stele: Pith parenchyma thin-walled, compact, forming a small cylinder in center not entered by bundles; vascular bundles concentric, numerous around perimeter of cylinder, farther apart but larger toward center; woody sheath prominent.

Summary: Cortex with prominent aerenchyma; vascular bundles strongly reinforced.

Histology of root (Plate XIX, fig. 2):

Primary cortex: Radius 5/6 radius of root; hypodermis, 2–3 rows of collenchyma;

parenchyma thin-walled, compact, with oval areas filled with very large parenchyma cells, 6–8 times the size of the round parenchyma (these areas show signs of breaking up and will probably give rise to air cavities); endodermis with thickened inner wall.

Stele: Parenchyma of small angular cells; bundles radial; a ring of large protoxylem cells lying within radial strands of metaxylem.

Summary: Mechanical tissue undeveloped; parenchyma prominent; tracheal vessels large.

Polygonaceae

Polygonum Muhlenbergii (Meisn.) Wats.

Habitat: Alluvial basin; muddy or dry places, rarely in shallow water.

Gross structure: Root thick, descending, tough, smooth; rootstocks branching; scaly, branching from root at different levels; horizontal, radial extension; perennial (Plate XV, fig. *a*).

Histology of rhizome (Plate XX, fig. 1):

Primary cortex: Depth 1/6 radius of rhizome, epidermis thin-walled; rectangular cells; hypodermis, several rows of cork; aerenchyma occupies 9/10 of cortical space.

Stele: 1/6 radius of rhizome; bundles collateral; bast forming a slender zone bordering phloem; phloem cells large, 6-sided; xylem with large tracheae; wood parenchyma 4- to 6-sided, compact, thick-walled.

Pith: Depth 2/3 radius of rhizome; homogeneous aerenchyma.

Summary: Vascular cylinder forming a narrow, compact zone; remainder of stem composed of aerenchyma.

Histology of root (Plate XX, fig. 2):

Primary cortex: Depth 1/4 radius of rhizome; hypodermal cork fills 1/5 of cortical area; large-celled, thin-walled parenchyma fills 4/5 cortical area; endodermis not distinct.

Stele: Collateral bundles; cone-shaped rays of old phloem surrounded by clusters of sclerenchyma extend into the cortical parenchyma, young phloem of rectangular cells adjacent to cambium; xylem forms a solid, pithless cylinder of large open tracheae and small, thick-walled, 4- to 6-sided wood parenchyma; the exarch character of bundle is shown in the small primary cylinder.

Summary: Parenchyma of cortex thin-walled with thick protective layer of cork; little mechanical tissue in cortex; relatively thick, compact wood parenchyma cells form a solid cylinder.

Ranunculaceae

Ranunculus delphinifolius Torr.

Habitat: Ponds, rooting in mud; stems and leaves floating.

Gross structure: Slender, fascicled roots; succulent; perennial.

Histology of root (Plate XX, fig. 3):

Primary cortex: Depth 4/5 radius of root; epidermal walls slightly thicker than walls of parenchyma; parenchyma cells large, oval to round, thin-walled, spongy; air cavities at intervals, endodermis walls of uniform thickness.

Stele: Vascular bundles radial; protoxylem and metaxylem distinct; tracheal tubes small.

Summary: Parenchyma prominent, thin-walled; vascular tissue poorly developed; mechanical tissue absent.

Saxifragaceae

Heuchera americana L.

Habitat: Moist to dry slopes; upland.

Gross structure: Thick, irregularly branched, tough rhizome with small secondary roots; perennial.

Histology of rhizome (Plate XXI):

Primary cortex: Depth 1/5 radius of root; hypodermis of thin-walled cork cells occupying 1/6 cortex; thick-walled parenchyma forms a band around phloem; endodermis not distinct.

Stele: Collateral bundles; phloem cells 4- to 6-sided; 3–4 strands of xylem extend from the center of the root radially outward, alternating with broad radii of thin-walled oval to square parenchyma cells.

Summary: Parenchyma prominent; mechanical tissue undeveloped; tracheal tissue proportionally small; cork well developed.

Rosaceae

Potentilla arguta Pursh.

Habitat: Dry, rocky, gravelly or alluvial soil.

Gross structure: Root tap, fibrous; thick, hard, tough; perennial.

Histology of root (Plate XXII, fig. 1):

Primary cortex: Depth 1/6 radius of root; cork hypodermis, thick-walled, occupies 1/2 of cortex; thick-walled, large-celled, angular parenchyma.

Stele: Radial bundles separated by broad rows of oval-celled parenchyma pith rays which radiate from the center of the root, forming fan-shaped terminal expansions the edges of which extend around the phloem; phloem thin-walled; 4- to 6-sided; xylem in several annular rings; wood parenchyma thick-walled; wood fibers are scattered among the wood parenchyma and form solid rings at termination of yearly growth; tracheae large.

Summary: Protective cork well developed; tracheae large and numerous; mechanical tissue in form of wood fibers prominent.

Leguminosae

Petalostemum candidum Michx.

Habitat: Dry prairie slopes; near base of hill commonly, while *P. purpureum* occupies ridges.

Gross structure: Root thick, tough; deep tap with prominent secondary roots; tubercled; perennial.

Histology of root (Plate XXII, fig. 2):

Primary cortex: Depth 1/6 radius of root; cork hypoderm occupies 1/4 of cortex; parenchyma large, angular, fairly thick-walled cells; endodermis not distinct.

Stele: Bundles collateral; clusters of bast lie within the older, and around the young, phloem; these tissues are enclosed by wood rays which pass as radii from the center of the root toward the bark, joining in arches around phloem; xylem with irregular clusters of large tracheae; few small wood parenchyma cells; compact, small wood fibers prominent.

Summary: Cork prominent; parenchyma little; mechanical tissue in form of bast and wood fibers, forming a tough, compact root.

Baptisia leucantha T. and G.

Habitat: Alluvial basin; moist, subject to drought; perennial.

Gross structure: Long, thick tap root with horizontal surface, fleshy branches; tough; tubercled; perennial.

Histology of root (Plate XXIII, fig. 1):

Primary cortex: Depth 1/2 radius of root; thin band hypodermal cork; large oval-celled starch parenchyma with scattered clusters of bast cells; endodermis not distinct.

Stele: Radial bundles; several concentric zones of bast cells laid down in clusters bordering the new phloem and lying within and around the older phloem; broad rows of wood rays of long parenchyma cells extending radially into cortex, spreading in fan-shaped areas of parenchyma; tracheae large, prominent; wood fibers conspicuous among wood parenchyma.

Summary: Cortex thick; mechanical tissue prominent; tracheae large, numerous; compact.

Desmodium illinoense Gray.

Habitat: Slopes; moist to dry.

Gross structure: Thick tap fibrous root; branched, tough; tubercled; perennial.

Histology of root:

Primary cortex: Depth 1/6 radius of root; narrow band of hypodermal cork; parenchyma thin-walled.

Stele: Radial bundle; wide rows of long, rectangular cells form wood rays which extend through xylem radially to bark where they form arches around phloem; annual rings prominent; wood parenchyma little; wood fibers abundant; tracheae large but few; large protoxylem and smaller metaxylem tissues of the primary xylem show the exarch character of the bundle.

Summary: Small cortex; prominent stele with much mechanical tissue in form of wood fibers; wood rays prominent; tracheae not conspicuous; compact.

Violaceae

Viola pedata L.

Habitat: Hill crests and slopes; dry, gravelly soil.

Gross structure: Rootstock short; erect; not scaly.

Histology of rhizome (Plate XXV, fig. 1):

Primary cortex: Depth 1/4 radius of root; hypodermal cork 2 or 3 rows; parenchyma large-celled, thin-walled; endodermis not distinct.

Stele: Vascular bundles distinct; collateral; separated by parenchyma rays which extend into the large pith cylinder; tracheae numerous, small; wood parenchyma thin-walled; pith parenchyma large-celled, round to oval; fairly compact.

Summary: Parenchyma prominent; mechanical tissue absent; tracheae small, numerous.

Gentianaceae

Gentiana puberula Michx.

Habitat: Moist slopes, upland mostly.

Gross structure: Slender rhizome bearing fascicles of relatively thickened roots.

Histology of root (Plate XXIII, fig. 2):

Primary cortex: Depth 1/2 radius of root; epidermal cells small, rectangular, thin-walled; 2–3 rows hypodermal collenchyma; large roundish parenchyma cells, fairly compact; endodermis not distinct.

Stele: Radial bundles, scattered clusters of phloem surrounded by large parenchyma cells; vascular cylinder small; tracheids numerous; remains of primary xylem show exarch character of primary bundle.

Summary: Parenchyma prominent, tracheae few; mechanical tissue absent.

Asclepiadaceae

Asclepias verticillata L.

Habitat: Alluvial basin; low prairie, moist soil.

Gross structure: Slender, radially extensive rhizome, bearing fascicles of thickened roots.

Histology of rhizome (Plate XXII, fig. 3):

Primary cortex: Depth 1/4 radius of rhizome; thick-walled, oval-celled parenchyma; endodermis not distinct.

Stele: Collateral bundles; phloem zone thin, unprotected, cells more or less crushed; xylem with few large tracheae; compact network of wood parenchyma;

small area of large-celled pith parenchyma into which project the remnants of the endarch xylem.

Summary: Parenchyma prominent; cortex relatively thick; mechanical tissue absent; tracheae few.

Labiatae

Monarda fistulosa L.

Habitat: Moist to dry slopes.

Gross structure: Elongated, slender, radially extensive rhizome, cross section square.

Histology of rhizome (Plate XXIV, fig. 1):

Primary cortex: Depth 1/4 radius of cylinder; epidermis of rectangular cells with uniformly thickened walls; hypodermal clusters of collenchyma cells in corners of square stem; parenchyma cells round, thin-walled; endodermis thin-walled.

Stele: Collateral bundles; vascular band very thin; 6-sided phloem cells protected by collenchyma; tracheae few; wood parenchyma small-celled; band of collenchyma separates xylem from pith; pith parenchyma of large, round, thin-walled cells.

Summary: Parenchyma prominent; mechanical tissue sparse; tracheae few.

Compositae

Vernonia fasciculata Michx.

Habitat: Alluvial basin; low wet ground.

Gross structure: Radially elongated, extensive rhizome with clusters of thick fascicled roots.

Histology of rhizome (Plate XXVII, fig. 1):

Primary cortex: Depth 1/2 radius of vascular cylinder; collateral bundles; epidermis of rectangular, uniformly thickened cells; hypodermis, 2–3 rows collenchyma; aerenchyma with branched sclerids at intervals; endodermis thin-walled.

Stele: Collateral bundles; phloem capped with clusters of bast; xylem fascicles separated by thick strands of rectangular-celled wood rays; tracheae few; wood parenchyma thick-walled, compact; wood fibers present; primary endarch bundle distinct; pith aerenchyma with scattered sclerids.

Summary: Aerenchyma prominent; tracheae inconspicuous; mechanical tissue fairly well developed.

Histology of root (Plate XXVII, fig. 2):

Primary cortex: Depth 5/6 of root; epidermis uniformly thickened; aerenchyma fills most of cortical space; endodermis thin-walled.

Stele: Radial bundles; small area of pith in center; tracheae few.

Summary: Aerenchyma prominent; cortex deep; vascular tissue limited.

Aster azureus Lindl.

Habitat: Hill crests and slopes.

Gross structure: Abbreviated rhizome, hard; clusters of roots form a thick fringe along the sides.

Histology of rhizome (Plate XXVI, fig. 2):

Primary cortex: Depth 1/7 radius of root; epidermis with horny, cuticularized edge; 3–4 rows hypodermal cork; zone of elongated collenchyma cells.

Stele: Bundles radial; thin-walled parenchyma bearing glands surrounds the phloem ring; bundles tipped with bast; xylem strands appear branched; traversed by annular rings of thin parenchyma; wood fibers strengthen xylem; broad wood rays of elongated parenchyma cells prominent; pith of large, rather thick-walled cells; primary endarch xylem distinct.

Summary: Mechanical tissue fairly prominent; xylem compact.

Artemisia ludoviciana (Nutt.) Riddell.

Habitat: Hillsides.

Gross structure: Slender, hard rhizome with clusters of filamentous roots.

Histology of rhizome (Plate XXIV, fig. 2):

Primary cortex: Depth 1/4 radius of root; cork 4 rows; parenchyma compact.

Stele: Bundles radial, distinct; phloem protected by patches of bast; vessels few; wood parenchyma cells large; bast sheath prominent; pith parenchyma compact.

Summary: Parenchyma well developed but compact; mechanical tissue conspicuous around bundle; xylem tissue sparse.

Antennaria plantaginifolia (L.) Richards.

Habitat: Dry hill crests and slopes.

Gross structure: Slender, radially extensive rhizomes with fringes of slender roots at their nodes.

Histology of rhizome (Plate XXVI, fig. 1):

Primary cortex: Depth 1/4 of radius of root; cork 1/4 of cortex; parenchyma thin-walled, rectangular; endodermis thin-walled.

Stele: Collateral bundles; phloem unprotected, rectangular cells; thick parenchyma rays separate the bundles; xylem with annular rings marked by layers of wood parenchyma at beginning of ring and thick-walled wood fibers at end; pith parenchyma large-celled, rather spongy.

Summary: Cork well developed; mechanical tissue in form of wood fibers prominent.

Liatris squarrosa Willd.

Habitat: Slopes, rather dry.

Gross structure: Stem corm-like, hard, flinty; somewhat scaly on surface; bears clusters of small filamentous roots.

Histology of corm (Plate XXVIII, fig. 2):

Primary cortex: Depth 1/6 radius of corm; cork 1/7 of cortex; cortical parenchyma of brick-like cells among which are a few sclerids; resin ducts present, bordered by 2 rows of thin parenchyma cells; endodermis not distinct.

Stele: Collateral bundles; clusters of sclerenchyma cells arranged around the perimeter of the phloem strands; parenchyma thin-walled; xylem in a series of clusters of annular growth; clusters of wood parenchyma and tracheae formed in spring; wood fibers formed in fall; xylem surrounded by fairly thick-walled, elongated parenchyma cells; resin ducts numerous throughout parenchyma; central pith of roundish cells.

Summary: Mechanical tissue prominent; resin ducts conspicuous; parenchyma compact.

Solidago canadensis L.

Habitat: Moist slopes; sometimes dry places.

Gross structure: Abbreviated rhizome with slender clusters of short roots.

Histology of rhizome:

Primary cortex: Depth 1/6 radius of rhizome; hypodermis of mechanical cells; parenchyma rather thick-walled; endodermis not distinct.

Stele: Collateral bundles; points of bast above tips of phloem; xylem fascicles branched outside of the second annular ring; broad bands of wood parenchyma cells separate the bundles; zones of parenchyma cells are left in spring growth; wood fibers appear in bands in fall growth.

Summary: Mechanical tissue prominent; tracheae fairly large.

Histology of root:

Primary cortex: Depth 1/2 radius of root; thick band collenchyma; parenchyma fairly thick-walled; endodermal walls not thickened.

Stele: Radial bundles show exarch arrangement of primary xylem; mechanical tissue fills center of cylinder; tracheae large.

Summary: Cortex deep; conspicuous mechanical tissue; large tracheae.

Heliopsis scabra Dunal.

Habitat: Dry to moist slopes.

Gross structure: Tuber-like rhizome; rather short; radial extension; bears clusters of small roots.

Histology of rhizome (Plate XXV, fig. 2):

Primary cortex: Depth 1/9 radius rhizome; hypodermal collenchyma; scattered sclerenchyma among the elongated parenchyma; endodermis walls of uniform thickness.

Stele: Collateral bundles; xylem fascicles branched above the first annual ring; broad rows of rectangular-formed wood rays separating the bundles or branches thereof; tracheae numerous; wood fibers prominent; primary endarch xylem distinct; pith parenchyma cells hexangular.

Summary: Mechanical tissue prominent; structure of stem compact.

Lepachys pinnata (Vent.) T. & G.

Habitat: Slopes; moist to dry.

Gross structure: Abbreviated rhizome; hard; clusters of slender roots.

Histology of rhizome (Plate XXVIII, fig. 1):

Primary cortex: Depth 1/6 radius of rhizome; parenchyma with clusters of sclerenchyma; endodermis not distinct.

Stele: Collateral bundles; xylem fascicles branched; separated radially and sometimes transversely by wood rays of parenchyma; tracheae few, small; wood parenchyma prominent; pith parenchyma with clusters of sclerids scattered through it.

Summary: Mechanical tissue abundant; tracheae small, few; parenchyma rays conspicuous.

Histology of root:

Primary cortex: Depth 1/2 radius root; parenchyma with sclerenchyma cells scattered through; endodermis not distinct.

Stele: Tracheae prominent; wood fibers abundant.

Summary: Mechanical tissue prominent; cortex deep.

Helianthus tuberosus L.

Habitat: Moist slopes.

Gross structure: Slender, radially extensive rhizomes; tuberous.

Histology of rhizome (Plate XXVII, fig. 3):

Primary cortex: Depth 1/4 radius of root; hypodermis of collenchyma; parenchyma large-celled, loose; resin glands scattered through parenchyma; endodermis not distinct.

Stele: Collateral bundles; phloem tipped with patches of sclerenchyma; tracheae few; woody parenchyma sparse, rather thick-walled; pith parenchyma of large, round cells, loose; resin glands scattered among pith.

Summary: Parenchyma prominent; mechanical tissue sparse.

Analysis of the Anatomy of Subterranean Organs

Of the twenty-six subterranean organs studied, fiften were upland plants and eleven were plants of the alluvial basin.

Alluvial basin species: *Typha latifolia*, *Phragmites communis*, *Spartina Michauxii*, *Panicum virgatum*, *Scirpus fluviatilis*, *S. validus*, *Iris versicolor*, *Polygonum Muhlenbergii*, *Ranunculus delphinifolius*, *Vernonia fasciculata*, *Asclepias verticillata.*

Upland species: *Heuchera americana*, *Potentilla arguta*, *Petalostemum candidum*, *Baptisia leucantha*, *Desmodium illinoense*, *Gentiana puberula*, *Asclepias verticillata, Monarda fistulosa, Aster azureus, Liatris squarrosa, Solidago canadensis, Heliopsis scabra, Helianthus tuberosus, Monarda fistulosa, Artemisia ludoviciana.*

The terminal or absorbing roots of four plants were studied and others were examined. The structure of the four roots drawn is typical and may be summarized as having a deep cortex (in this case 4/5–5/6 the radial depth of the stem) of aerenchyma or loose parenchyma, only a trace of, or no, mechanical tissue, and a variable vascular tissue with reference to the size and number of tracheae. It is evident that the function of these roots is absorption only. There is usually a little mechanical tissue in the upland species and a little more specialization of the vascular tissue.

Ranunculus multifidus has entirely a fibrous root and retains the primitive root character to maturity. It has no mechanical tissue.

Of the root structures studied, six were of upland plants and one of an alluvial basin plant. (Most of the prominent alluvial basin plants have rootstocks). Here parenchyma, found only in the cortex, was compact, reduction in space being supplemented by the presence of cork, collenchyma, or mechanical tissue. Reduction or absence of mechanical tissue was

Comparison of Tissues of the Subterranean Organs

Roots

Name	Habitat	Root	Radial Depth Cortex	Parenchyma	Mechanical Tissue	Vascular Tissue
Typha latifolia	Al. bas.	Terminal	4/5	Aerenchyma	None	Fairly prominent
Phragmites communis	Al. bas.	Terminal	5/7	Aerenchyma	Trace	Distinct; not prominent
Iris versicolor	Al. bas.	Terminal	5/6	Aerenchyma	Moderate	Fairly prominent
Vernonia fasciculata	Al. bas.	Primary	1/2	Aerenchyma prominent; parenchyma	Moderate	Limited; tracheae few
Ranunculus delphinifolius	Al. bas.	Main fibrous	4/5	Aerenchyma	None	Limited
Polygonum muhlenbergii	Al. bas.	Primary	1/4	Spongy cork	Moderate	Tracheae numerous
Lepachys pinnata	Upland	Terminal	1/2	Fairly compact	Slight	Tracheae prominent
Potentilla arguta	Upland	Primary	1/6	Compact	Prominent	Tracheae large, numerous
Desmodium illinoense	Upland	Primary	1/6	Slight cork	Prominent in stele	Tracheae large, few
Petalostemum candidum	Upland	Primary	1/6	Thick-walled cork	Prominent	Tracheae large, numerous
Gentiana puberula	Upland	Primary	1/2	Prominent	Absent	Tracheae few
Solidago canadensis	Upland	Secondary	1/2	Parenchyma thick-walled	Prominent	Tracheae large
Baptisia leucantha	Al. bas.	Primary	1/2	Prominent	Moderate	Tracheae few

Subterranean Stems

Name	Habitat	Subterranean Stem	Radial Depth Cortex	Parenchyma	Mechanical Tissue	Vascular Tissue
Typha latifolia	Al. bas.	Rhizome	1/2	Aerenchyma	Slight in vas. bund. sheath	Fairly prominent
Phragmites communis	Al. bas.	Rhizome	1/2	Aerenchyma	Slight	Few bundles
Spartina Michauxiana	Al. bas.	Rhizome	3/7	Slight	Prominent	Vas. bundles large, numerous tracheae
Panicum virgatum	Al. bas.	Rhizome	2/9	Slight	Prominent	Vas. bundles large
Scirpus fluviatilis	Al. bas.	Rhizome	1/2	Aerenchyma	In vas.bund. sheaths	Vas. bundles numerous
Scirpus validus	Al. bas.	Rhizome	1/3	Aerenchyma	In vas.bund. sheaths	Prominent
Iris versicolor	Al. bas.	Rhizome	1/3	Aerenchyma	In vas.bund. sheaths	Prominent
Polygonum Muhlenbergii	Al. bas.	Rhizome	1/6	Aerenchyma	Absent except few wood cells	Slight
Asclepias verticillata	Al. bas.	Rhizome	1/4	Prominent	Absent	Tracheae few
Monarda fistulosa	Al. bas.	Rhizome	1/4	Prominent	Slight	Tracheae few
Heuchera Americana	Upland	Rhizome	1/5	Prominent	Absent	Tracheae; tissue small
Viola pedata	Upland	Rhizome	1/4	Prominent	Absent	Tracheae small, numerous
Aster azureus	Upland	Rhizome	1/7	Cork slight; parenchyma	Fairly prominent	Tracheae numerous
Antennaria plantaginifolia	Upland	Rhizome	1/4	Moderate	Prominent wood fibers	Tracheae small, inconspicuous
Liatris squarrosa	Upland	Corm	1/6	Compact	Prominent	Few tracheae; small
Heliopsis scabra	Upland	Rhizome	1/9	Slight	Prominent	Tracheae numerous
Lepachys pinnata	Upland	Rhizome	1/6	Slight	Prominent	Tracheae small, few
Artemisia ludoviciana	Upland	Rhizome	1/4	Prominent	Moderate	Few tracheae
Helianthus tuberosus	Upland	Rhizome	1/4	Prominent	Slight	Tracheae few

prominent in 66 percent of the roots. Tracheae were generally large and quite numerous.

The only thick root of the alluvial basin studied was that of *Baptisia leucantha*, which had prominent parenchyma, moderate mechanical tissue, and few tracheae.

The subterranean stems of ten alluvial basin and nine upland plants were examined. Of the alluvial basin plants, 65 percent had aerenchyma and only 10 percent reduced parenchyma. In only 10 percent was mechanical tissue prominent though present about the sheaths of monocotyledons. In the monocotyledons the vascular tissue was fairly prominent; in dicotyledons it was poorly developed.

In the stems of the upland plants there is considerable variation in the relative proportion of parenchyma, though in the plants of the drier habitats it was present in very slight degree. Mechanical tissue was prominent in most of the plants with large aerial portions, and usually absent in the low-growing plants with the exception of Helianthus which seems to be a non-conformist in both leaf and stem characters and lives by a code of its own. The tracheae were also variable in size and number. In this small group of plants no correlation could be seen between the number of tracheae and the number of leaves. The presence of thick-walled cells not only serves as a reinforcing character but no doubt tends to preserve turgor and to protect water-conducting tissues against loss of water.

SUMMARY

A study of the minute anatomy of subterranean organs of prairie plants shows:

(1) There is a tendency to the production of prominent mechanical tissue in plants of dry habitats and reduction of parenchymatous tissue.

(2) In moist habitats the proportion of parenchymatous tissue is prominent. Aerenchyma is abundant in swamp plants.

(3) The vascular tissue is variable in quantity, seemingly more or less subject to systematic variation.

The subterranean stem is predominant as an equivalent of the primary root, especially in moist lowland regions. It is more efficient than the root in propagation. Primary roots which show secondary thickening resemble stems in their concentric manner of expansion. The stem has an area of pith which serves as a reservoir for water and hence increases its efficiency for radial distribution.

I wish to acknowledge the helpful comment of Dr. L. H. Pammel, Dr. J. N. Martin, and Miss Charlotte M. King in the preparation of this paper.

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EXPLANATION OF PLATES XV–XXVIII

A, aerenchyma; *B*, bast; *C*, collenchyma; *CM*, cambium; *CK*, cork; *EN*, endodermis; *EP*, epidermis; *P*, parenchyma; *PH*, phloem; *RC*, resin canal; *SC*, sclerenchyma; *RH*, root hair; *T*, tracheae; *X*, xylem; *PX*, protoxylem; *MX*, metaxylem; *WF*, wood fibers; *WP*, wood parenchyma; *WR*, wood rays; *VB*, vascular bundle.

The figures were made with the aid of a camera lucida. The compound microscope was a Spencer, Oculars 5× and 10× and objectives 4 and 16, with a standard tube length, were used in various combinations.

PLATE XV

Subterranean Systems of Water Plants

FIG. *a*. *Polygonum Muhlenbergii.*
FIG. *b*. *Phragmites communis.*
FIG. *c*. *Scirpus fluviatilis.*
FIG. *d*. *Typha latifolia.*
FIG. *e*. *Iris versicolor.*
FIG. *f*. *Sagittaria latifolia.*
FIG. *g*. *Scirpus validus.*
FIG. *h*. *Spartina Michauxiana.*

PLATE XVI

FIG. 1. *Typha latifolia*, rhizome, × 75.
FIG. 2. *Typha latifolia*, root, × 93.

PLATE XVII

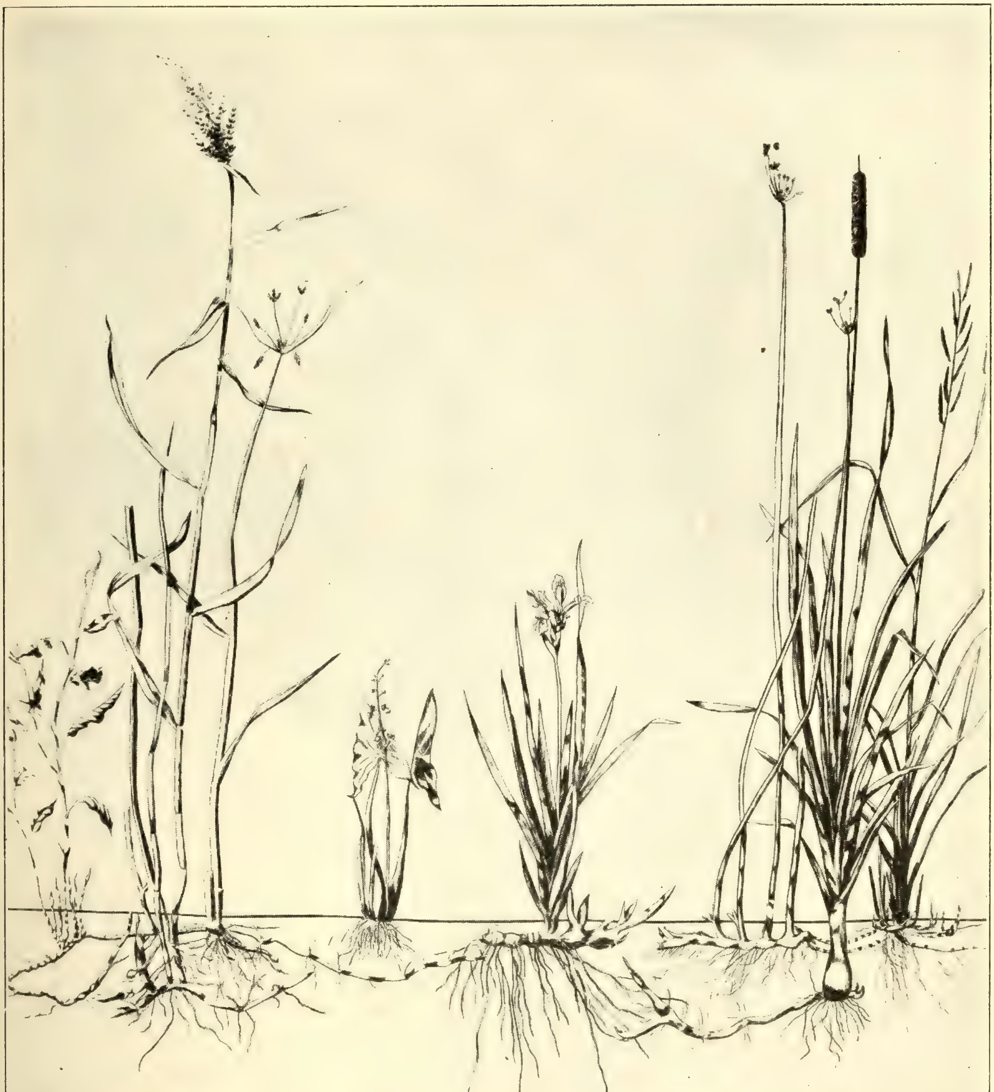
FIG. 1. *Phragmites communis*, rhizome, × 150.
FIG. 2. *Phragmites communis*, root, × 115.

PLATE XVIII

FIG. 1. *Spartina Michauxiana*, rhizome, × 125.
FIG. 2. *Panicum virgatum*, rhizome, × 102.

PLATE XIX

FIG. 1. *Iris versicolor*, rhizome, × 50.
FIG. 2. *Iris versicolor*, root, × 75.
FIG. 3. *Scirpus fluviatilis*, rhizome, × 50.



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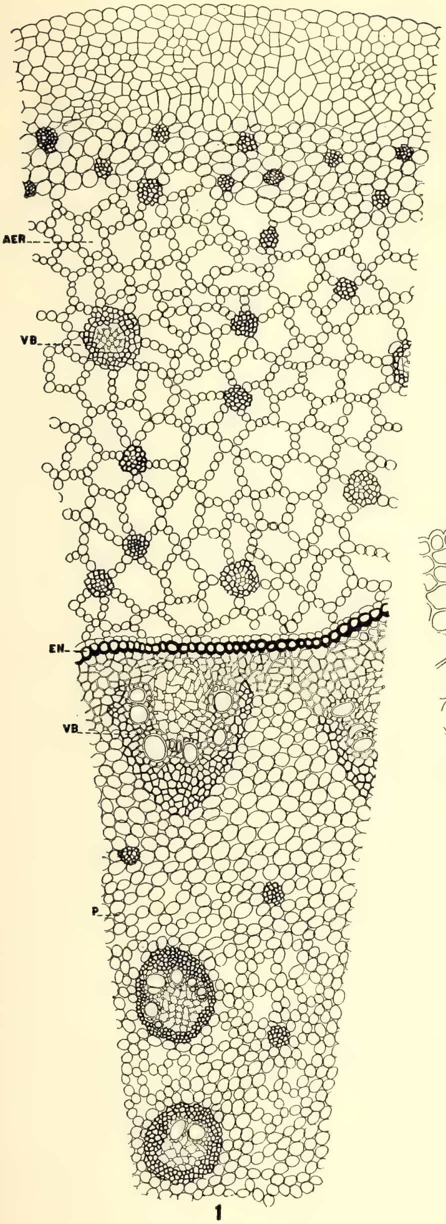
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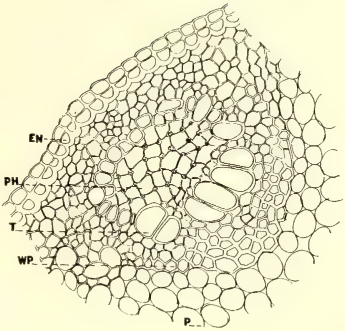

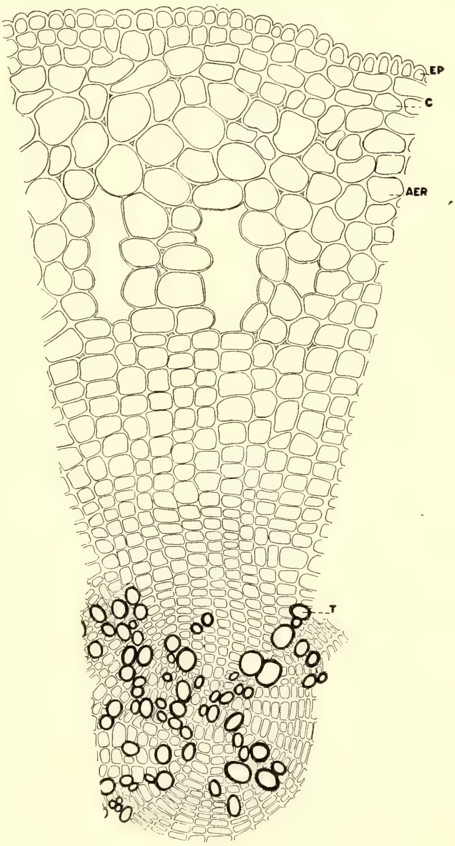

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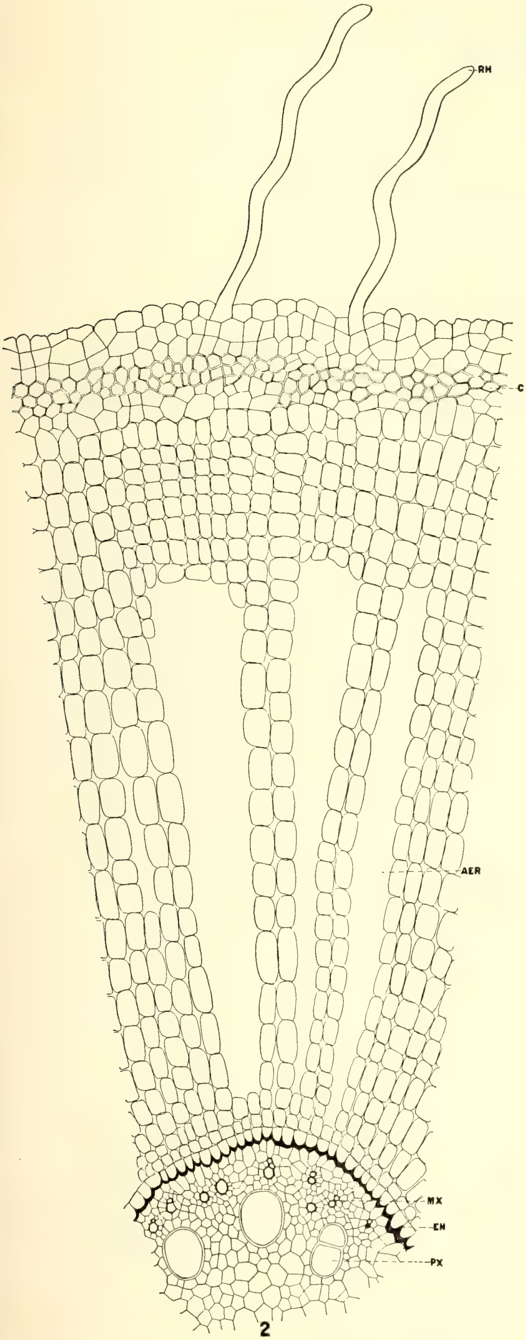
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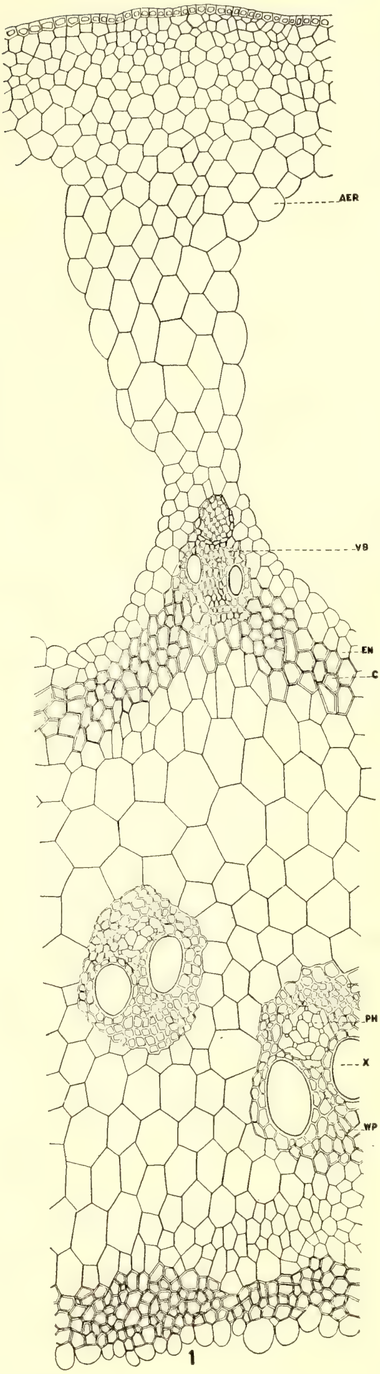
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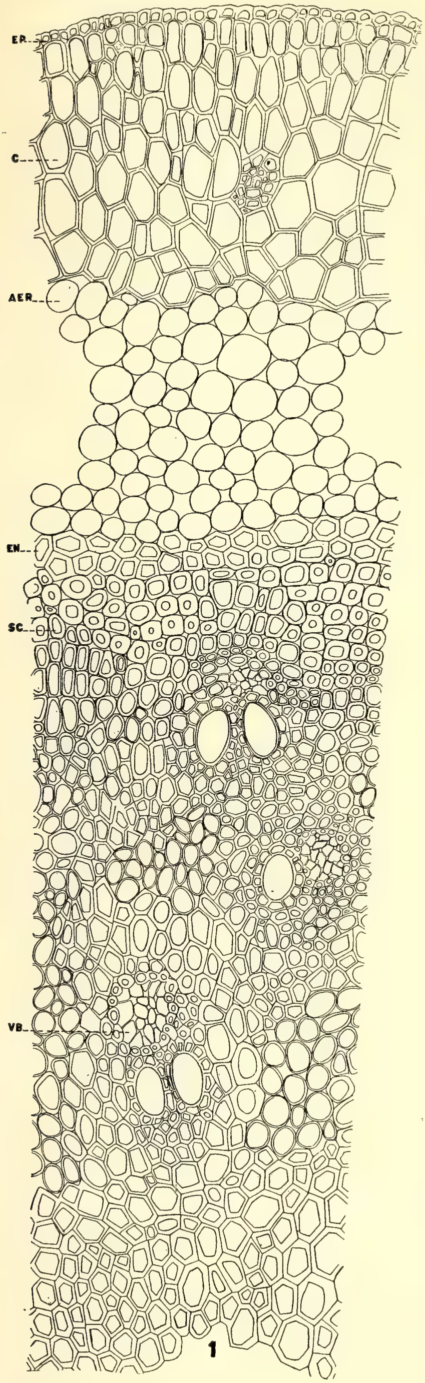
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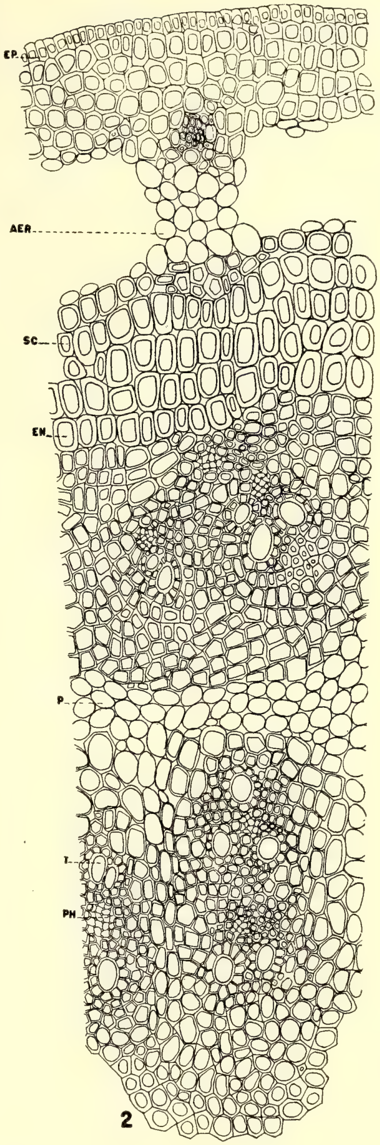
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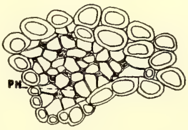

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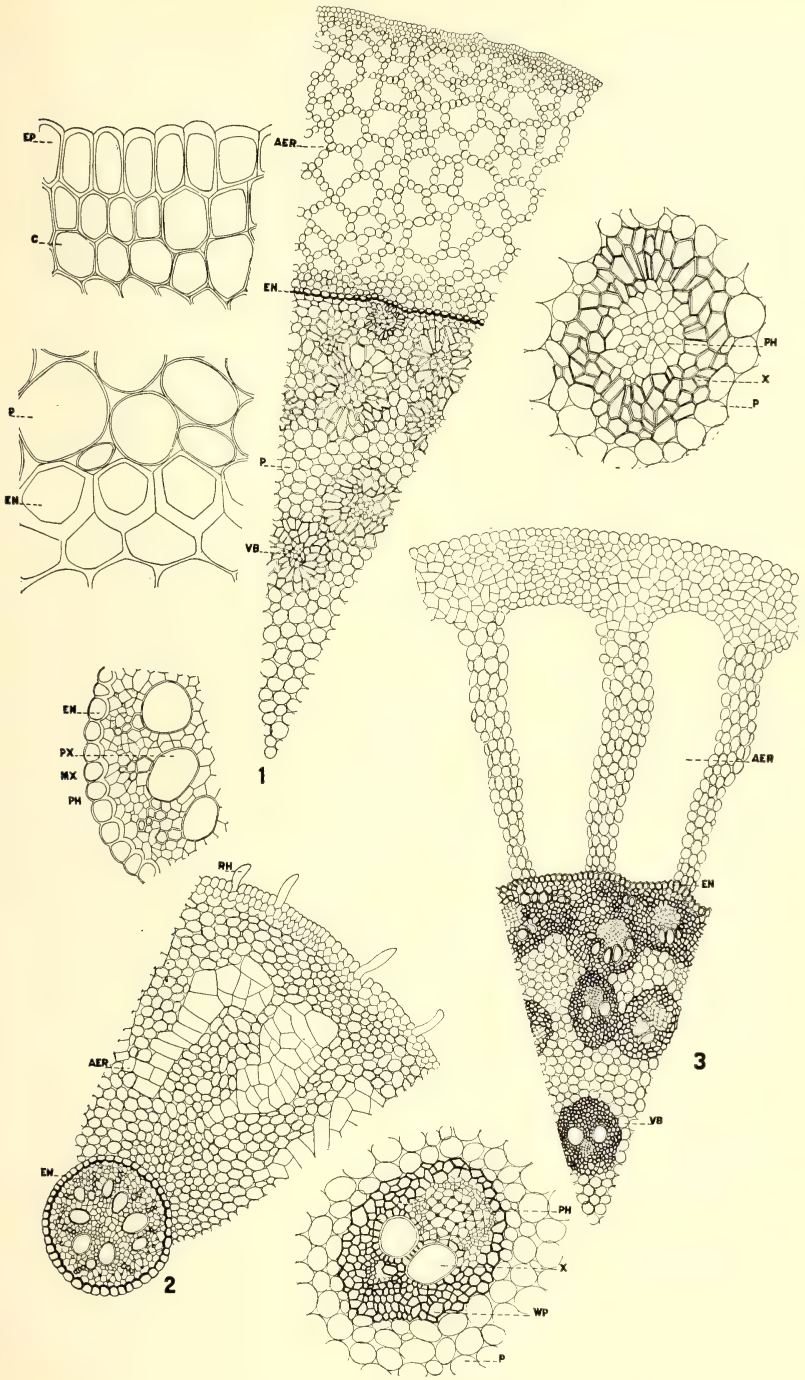
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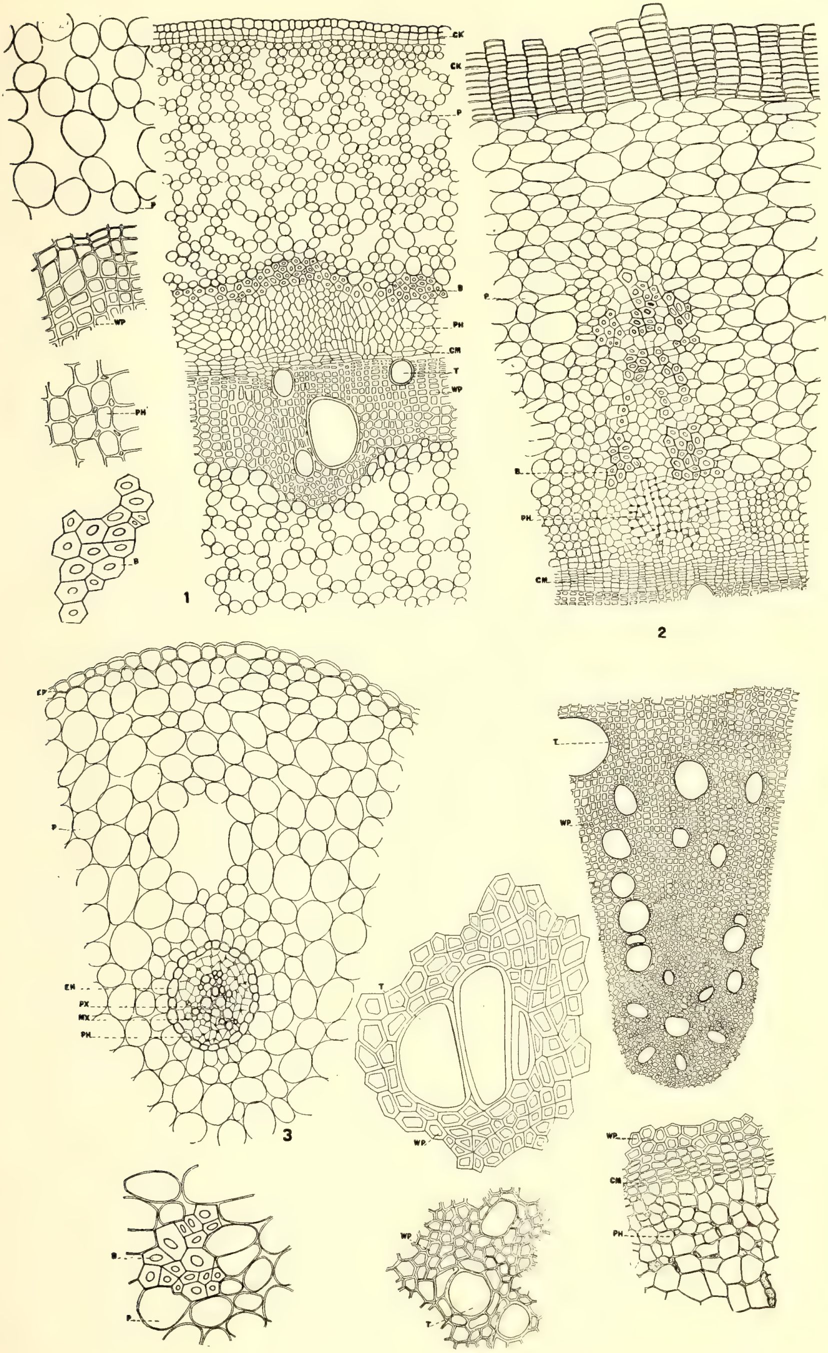
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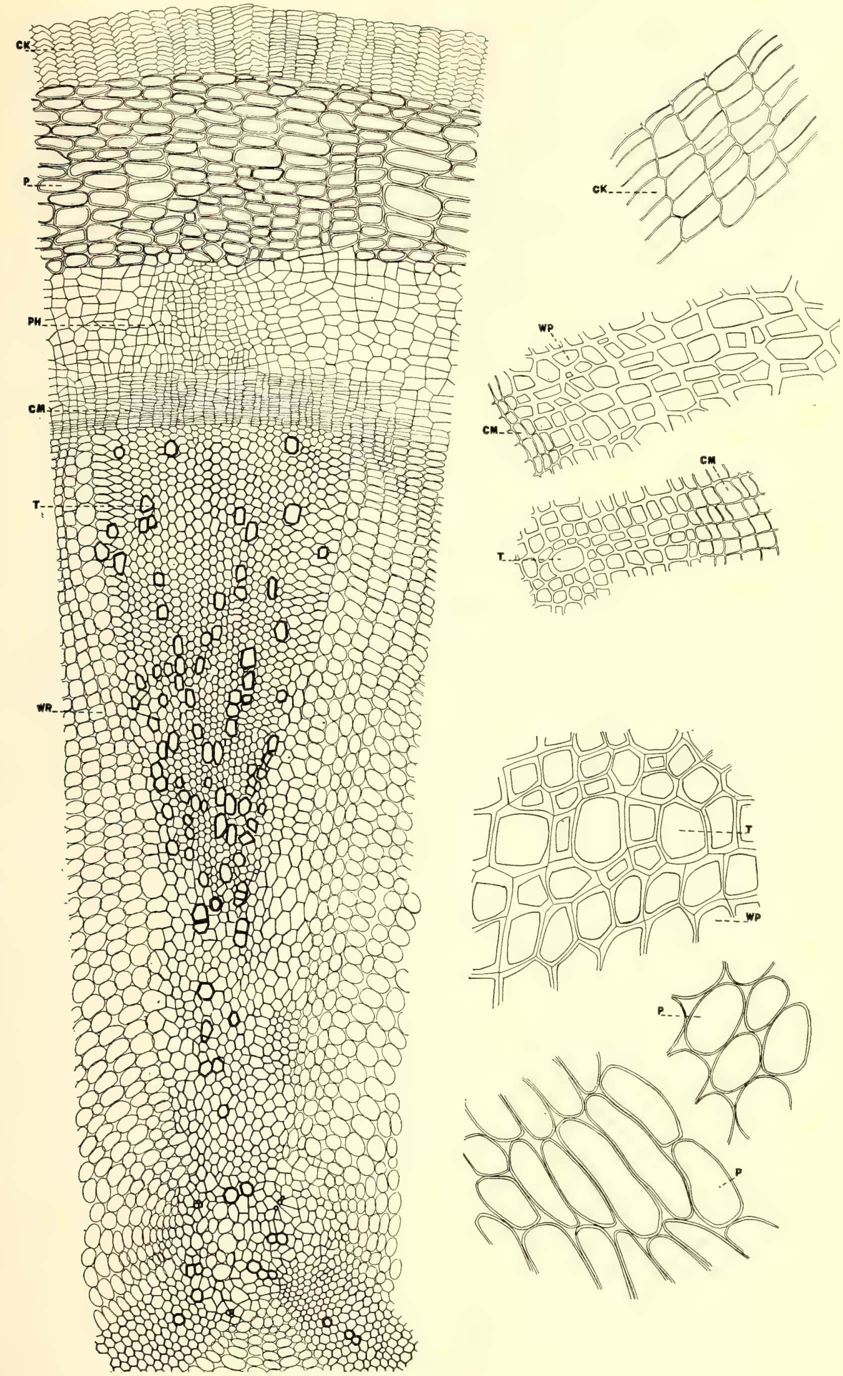
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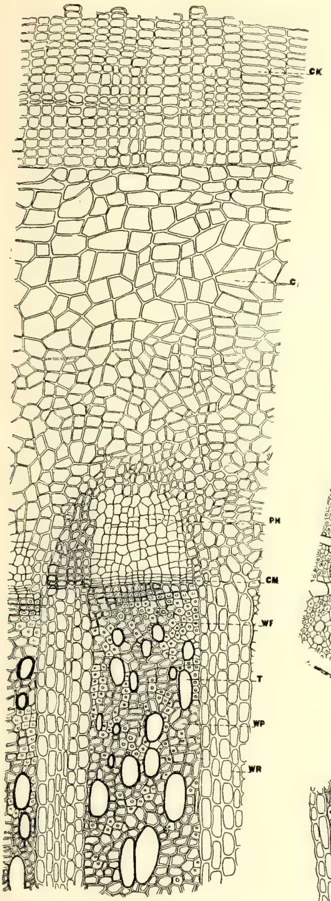
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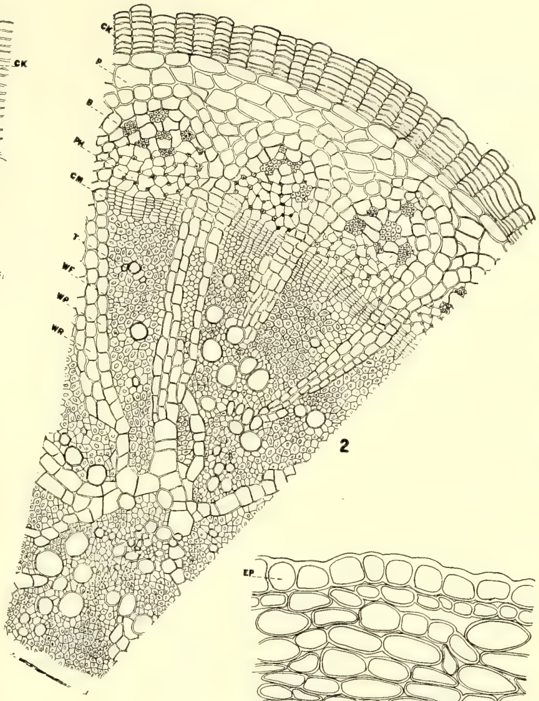
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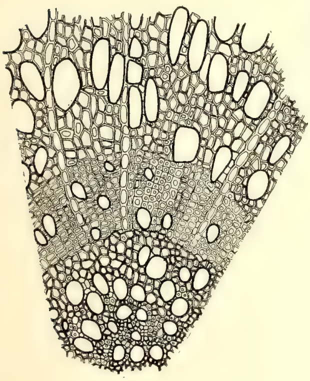

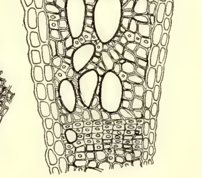

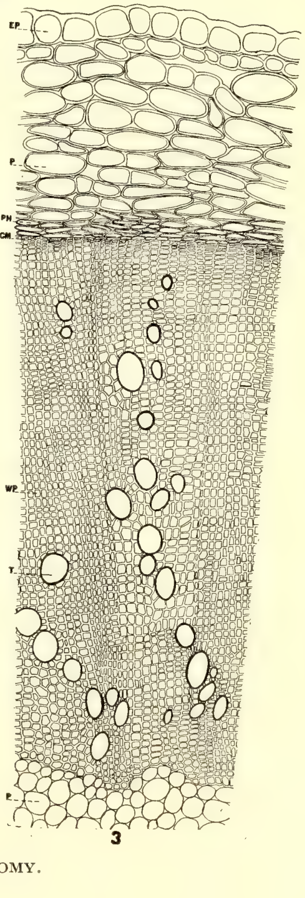
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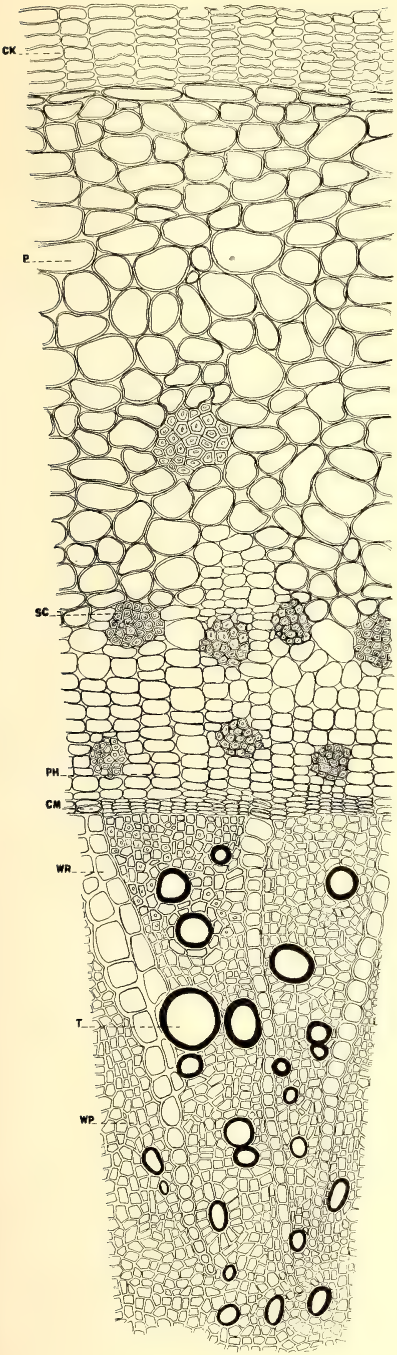
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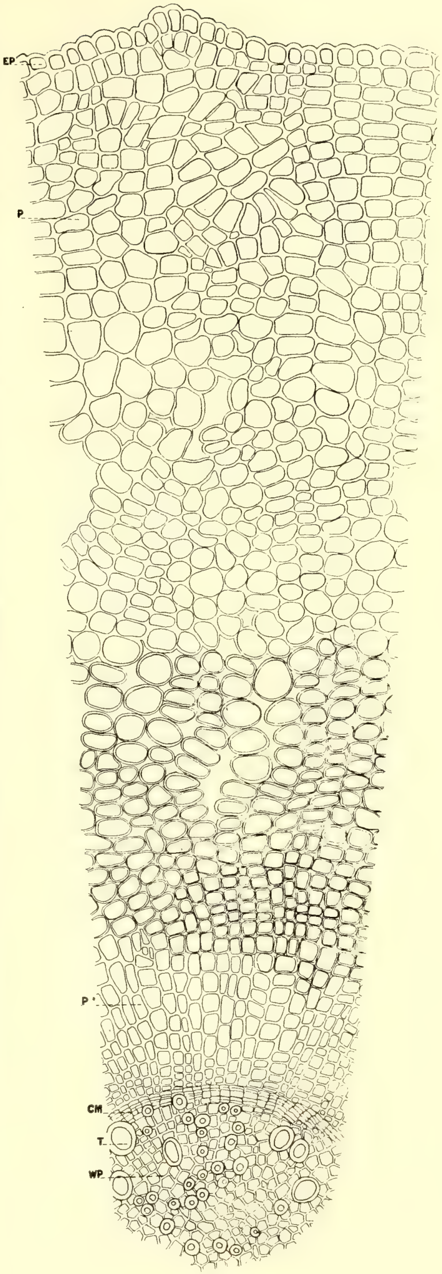

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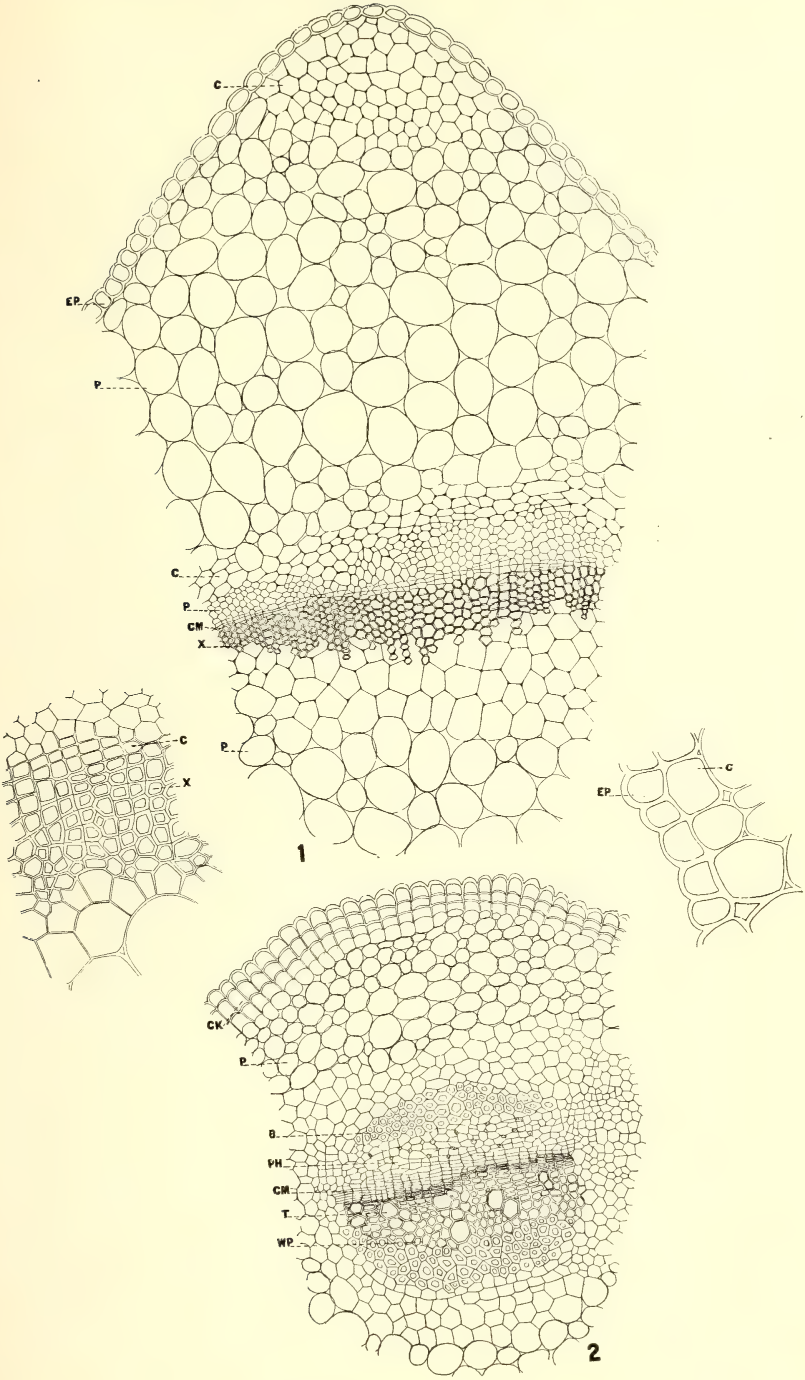
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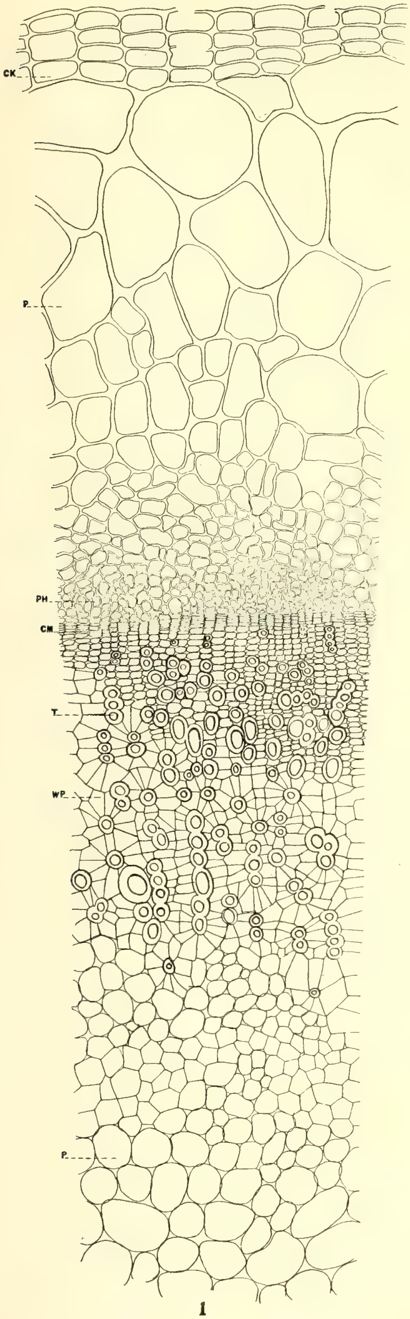
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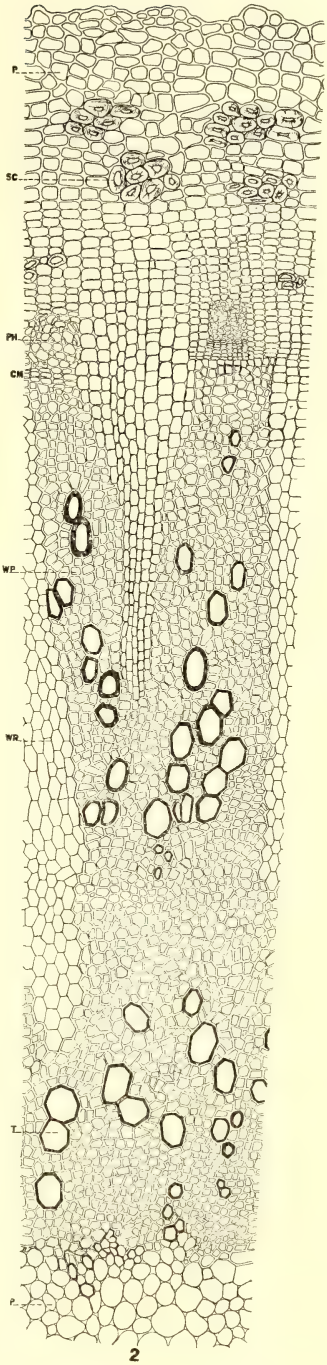
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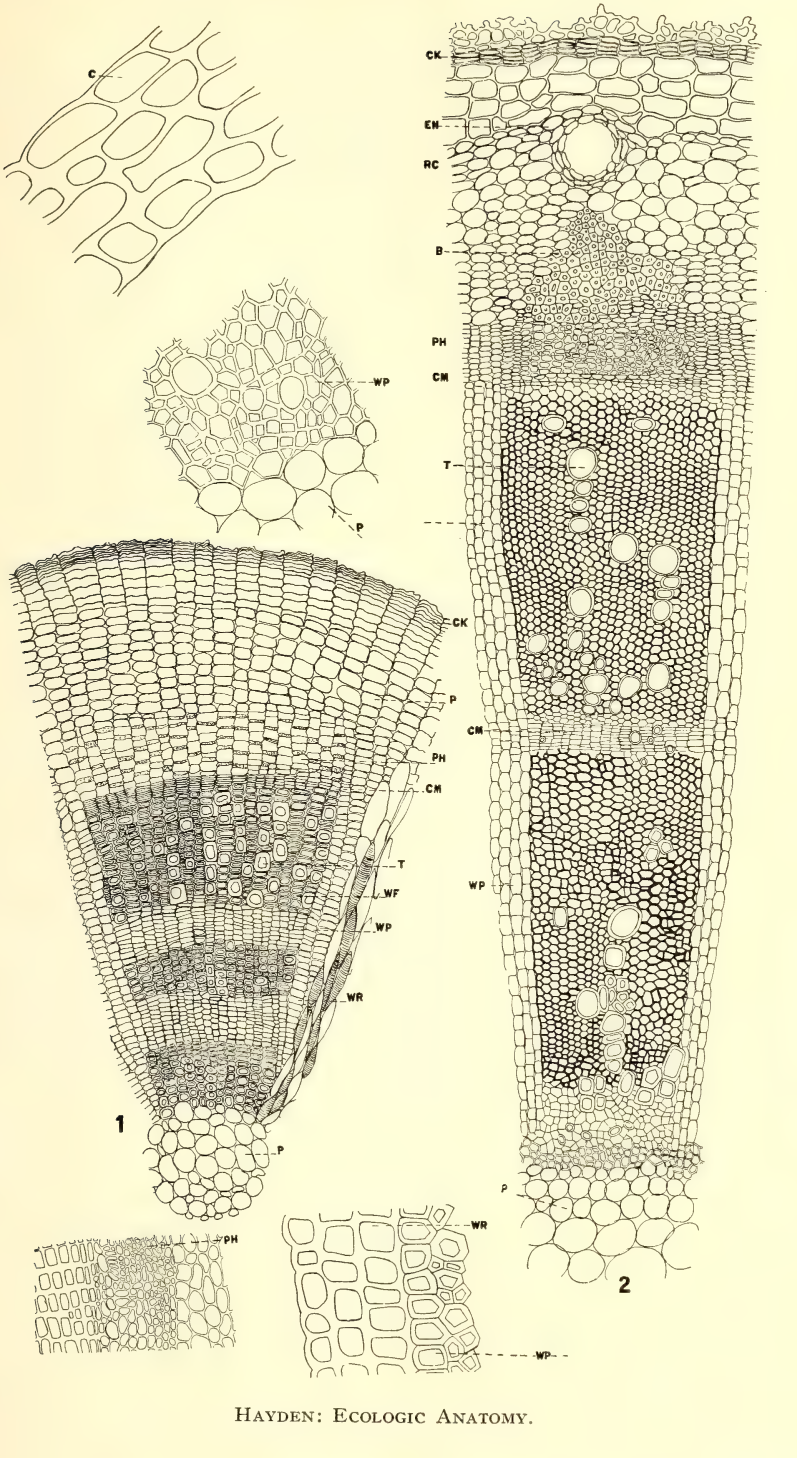
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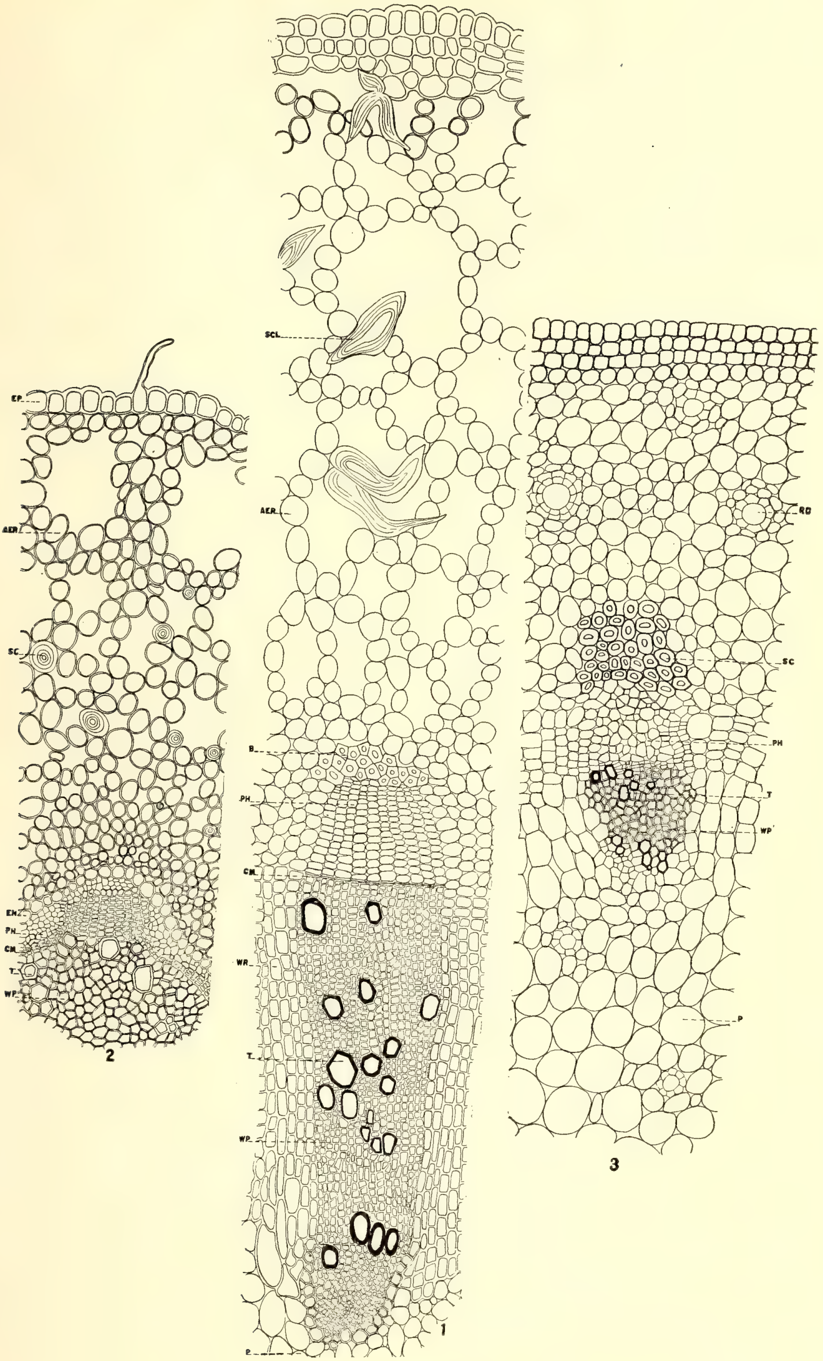
Hayden: Ecologic Anatomy.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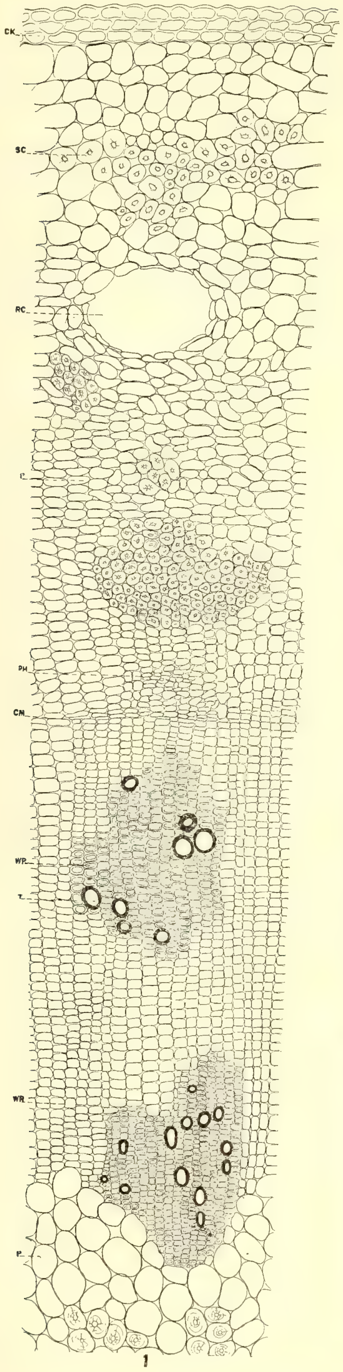
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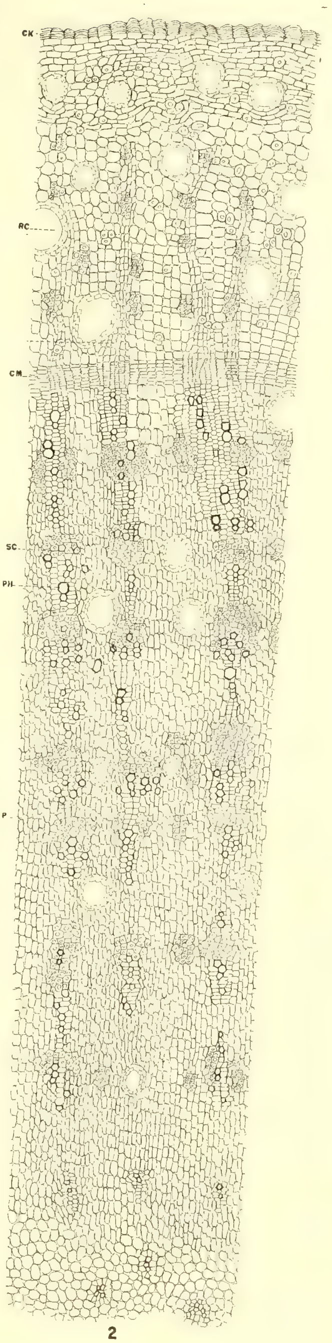
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HAYDEN: ECOLOGIC ANATOMY.

Plate XX

Fig. 1. *Polygonum Muhlenbergii*, rhizome, × 45.
Fig. 2. *Polygonum Muhlenbergii*, root, × 45.
Fig. 3. *Ranunculus delphinifolius*, root, × 100.

Plate XXI

Fig. 1. *Heuchera americana*, rhizome, × 80.

Plate XXII

Fig. 1. *Potentilla arguta*, root, × 100.
Fig. 2. *Petalostemum candidum*, root, × 75.
Fig. 3. *Asclepias verticillata*, rhizome, × 75.

Plate XXIII

Fig. 1. *Baptisia leucantha*, root, × 150.
Fig. 2. *Gentiana puberula*, root, × 150.

Plate XXIV

Fig. 1. *Monarda fistulosa*, rhizome, × 125.
Fig. 2. *Artemisia ludoviciana*, rhizome, × 50.

Plate XXV

Fig. 1. *Viola pedata*, rhizome, × 75.
Fig. 2. *Heliopsis scabra*, rhizome, × 90.

Plate XXVI

Fig. 1. *Antennaria plantaginifolia*, rhizome, × 165.
Fig. 2. *Aster azureus*, rhizome, × 110.

Plate XXVII

Fig. 1. *Vernonia fasciculata*, rhizome, × 60.
Fig. 2. *Vernonia fasciculata*, root, × 90.
Fig. 3. *Helianthus tuberosus*, rhizome, × 76.

Plate XXVIII

Fig. 1. *Lepachys pinnata*, rhizome, × 85.
Fig. 2. *Liatris squarrosa*, corm, × 16.

STUDIES CONCERNING THE EVOLUTIONARY STATUS OF POLYCOTYLEDONY

John T. Buchholz

The question which condition is more primitive, polycotyledony or dicotyledony, has been very widely discussed and is a very old subject in botanical literature. It is generally believed that one of these has been derived from the other, and for more than seventy years botanical opinion has been divided on the question. On the basis of anatomy, Duchartre, in 1848, supported the thesis which had been previously announced, that polycotyledony has been derived from dicotyledony, but this view was not accepted by Sachs in his text-book (1882). Masters in 1891 supported the views of Sachs, and Dangeard supported the older view of Duchartre. More recently, Hill and DeFraine (6), after the study of the vascular anatomy of many conifer seedlings, also reached the conclusion that polycotyledony has been derived from dicotyledony by a splitting of the cotyledons.

Hill and DeFraine find that in most instances there is a single vascular strand in the cotyledon. They classify as whole-cotyledons those in which this strand undergoes bifurcation accompanied by a rotation of the xylem to bring the protoxylem in the exarch position as it forms a single root pole; as half-cotyledons those in which the strands from two cotyledons combine during transition to form one root pole; and as subsidiary-cotyledons those in which the strand fuses with another above the transition region. The existence of intermediate stages leads these authors to infer that "a subsidiary seed-leaf may, in the course of events, be promoted, as it were, to the rank of a half-cotyledon; while a half-seed-leaf may be raised to the dignity of a whole-cotyledon." In addition to this, they find that occasionally leaves from the plumule may become displaced and added to the cotyledonary node.

The argument of Hill and DeFraine rests further upon the existence of cotyledons with double strands, partially divided cotyledons, and numerous similar abnormalities. The theory is more plausible when applied to the Taxineae, Podocarpineae, and other forms with few cotyledons, but becomes very difficult to explain for the more extremely polycotyledonous Abietineae. While these authors make use of the external anatomy in their hypothesis, they give us no explanation for the origin of the cotyledonary tubes which they found in many instances among twenty species—nearly one third of the number investigated.

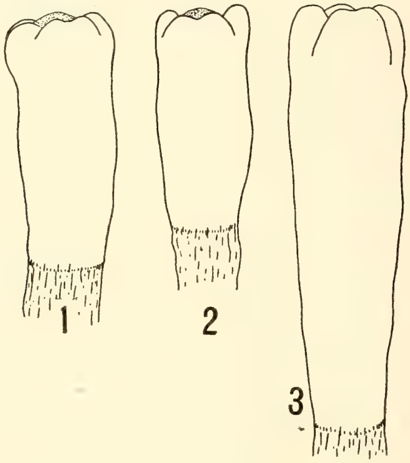
On the other hand, assuming that when two cotyledons fuse they form double cotyledons, which later lose their double nature, and that the cotyledonary tubes are the direct result of the incomplete fusions, it is just as easy, on the basis of the anatomical facts presented by Hill and DeFraine, to pass from polycotyledony to dicotyledony as *vice versa.* In support of the view that polycotyledony is primitive, Sister Helen Angela (1) has prepared a series of diagrams based on vascular anatomy, which show all stages in a series of intergrading forms from the polycotyledonous to the dicotyledonous condition, in Coniferales as well as in Cycadales. But the evidence mustered by vascular anatomy or by the study of the occasional freaks in which the cotyledons seem to be partly divided, is merely proof that one condition has without doubt arisen from the other, and leaves us with no very positive clue as to which course evolution has been taking—which condition has actually given rise to the other.

The study of fossil material has frequently furnished us with a definite record of the past history of a group. The fact that the embryos that have thus far been found in the most primitive gymnosperm seeds, those of the Benettitales, are dicotyledonous has led to a rather widespread impression that geological proof establishes dicotyledony as the more primitive condition. On the other hand, it is now well known that the cycad line, of which the Benettitales are the Mesozoic representatives, has been distinct from the conifer line since Paleozoic time. The Cordaitales were probably the ancestors of the whole conifer line which includes Ginkgoales as well as Coniferales. The cycad line, on the other hand, has been derived from the Cycadofilicales which also existed in Carboniferous time along with the Cordaitales, and these two phylogenetic lines of seed plants have been distinct from each other since very early times. Thus the dicotyledonous embryos of the Bennettitales do not represent the ancestral condition from which the conifers were derived, and we have no knowledge of the ancient conifer embryos from a study of fossil material. Coulter and Chamberlain (5), who do not accept the conclusions of Hill and DeFraine as final but favor the opposite view, point out that "probably our oldest group of Coniferales, older even than the Cycadales and Benettitales with which we are acquainted, is the extreme illustration of polycotyledony, while the youngest of the Coniferales are dicotyledonous or nearly so." Until some paleobotanist describes embryo-bearing seeds of the most ancient conifers or Cordaitales, we shall need to look to our living material for our information, or content ourselves with a philosophical discussion of the question.

Investigation

This paper is the result of a study of the ontogeny of the cotyledons in various living species of conifers, in the hope that this evidence may reveal modern evolutionary tendencies and afford a safe criterion from which to determine in what direction this particular evolution of the cotyledons has been proceeding. When such evidence is not taken from a single isolated species, but is found among representatives of different genera taken more or less at random and is in harmony with all the known facts, it establishes a safe morphological basis for conclusions.

The cotyledons develop in the other conifers in practically the same way as the writer has described for Pinus (2). As pointed out there, the stem tip primordium appears before the primordia of the cotyledons. It consists of a small protuberance at the apex of the dome which crowns the cylindrical cell mass of the undifferentiated embryo. Meanwhile, the whole embryo mass enlarges, and soon a circle of cotyledon primordia appear very nearly simultaneously, surrounding the stem tip. These primordia are little protuberances like the stem tip primordium and they soon elongate to form the cotyledons. They are separate and distinct from each other when they first appear, and their number is not constant but varies, much as does the number of cotyledons that are found in the matured embryo.



FIGS. 1–3. Embryos of *Pinus Banksiana* which are occasionally found, showing cotyledonary fusion in the primordial stage. × 32.

The primordia are formed long before there are any vascular strands. The latter are formed only some time after the cotyledons have begun to elongate. Therefore, evidence based on the origin of the primordia and of the cotyledons from these primordia has much greater morphological value than the study of the later appearing vascular structures, and has in addition the advantage of being capable of showing a definite recapitulation of the more primitive condition.

The writer has published evidence of cotyledonary fusions in the primordial stage of *Pinus Banksiana* (2). This species has a small number of cotyledons, ranging between three and six, the usual number being four. Here the number of primordia is sometimes greater than the number of cotyledons, and a number of instances were found which showed various stages in the fusion of two cotyledon primordia to form single broad cotyledons, some of which are shown in figures 1, 2, and 3. In this species, the fusion is not always complete until after the cotyledons have begun to elongate, thus making its recognition as a process of fusion very certain.

Since this work on the pine embryo, the writer has been studying the embryos of a number of other conifers. Several species among other genera were found in which the cotyledons fuse in the primordial stages much as they do in Pinus, while still others were studied in which this performance has been completely eliminated from their ontogeny. However, no instances were found in which a small number of cotyledon primordia gave rise to a larger number of cotyledons.

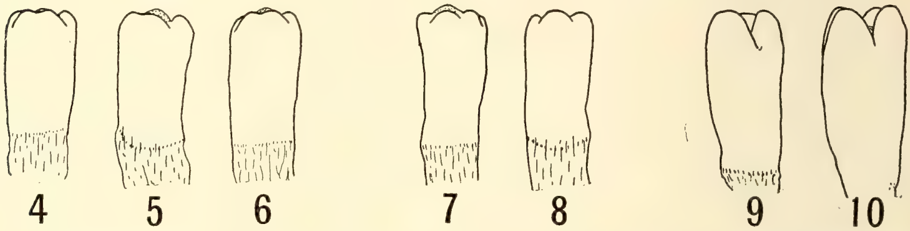
Another good example of cotyledonary fusion may be found in *Picea mariana*. The material used in this study was sent to the Hull Botanical Laboratory from northern Wisconsin (Oneida County) about the first of August 1917. Through the kindness of Dr. George D. Fuller, the writer secured a number of cones from this collection. The specimen included the upper three feet of a black spruce tree unusually well laden with cones. Only one collection was available, and when the seeds proved to contain embryos in the cotyledon primordia stage, several hundred of them were dissected out under a binocular dissecting microscope and preserved in formalin-alcohol.

The drawings were made under the low power of a compound microscope with an Abbe camera lucida. Figures 1–3 and figures 24 and 25 are from stained permanent preparations; all of the remaining figures were made from the unstained embryos. In the figures, the stem tip primordium was shaded slightly so that it might be easily distinguished from the primordia of the cotyledons. The embryos are turned with their stem tips up because they are more easily handled in this position under the binocular dissecting microscope, the suspensor furnishing a convenient means of tilting, turning them over, and otherwise handling them without injuring their tips.

From the first, the writer was impressed by the large number of instances in which the sinus between two adjoining primordia seemed to be disappearing, resulting in a single broad cotyledon. By reference to the accompanying figures 4, 5, and 6, it will be noted that these primordia fuse very early, before there is any well marked elongation of the cotyledons. In many cases this fusion was so striking that it could easily be noticed, but in others it was less marked. To see these primordia distinctly, it was nearly always necessary to study the contour of the embryos as they were held in a slanting position and turned on their long axis with the apex pointing away from the observer. This avoids confusing the stem tip with the cotyledons, and makes young and obscure cases appear more distinct.

The writer realized that some of his readers would be inclined to take exception to conclusions based alone on appearances, as shown by the drawings of these embryos. Figures 4 and 6 might be looked upon as showing broad primordia in a state of division, since in this species the fusion occurs before the cotyledons begin to elongate and is therefore much more rapid than in *Pinus Banksiana.* In the latter case, where the period of fusion usually lasts longer, this error of a reversed interpretation could not so easily be made. Furthermore, the variations that occur in the cotyledon number make it more difficult to recognize either a fusion tendency or the opposite condition.

To prove that these are cotyledonary fusions in Picea, it is necessary to rely in part on the statistical method. If, on an average, the younger embryos can be shown to have a larger number of cotyledon primordia than the average number of cotyledons in the older embryos, then there is unquestionably a reduction in their number, and the double primordia are in the act of fusion. If, on the other hand, the older embryos have a greater number of cotyledons than the number of primordia, or, counting these double primordia as two, if the resulting cotyledons are of the same average number as the primordia, then these double primordia must be cases of splitting cotyledons.



Picea Mariana. FIGS. 4–6 are from lot B showing double cotyledon primordia that are undergoing fusion. FIGS. 7–8, embryos from lot C with no fusing primordia. FIGS. 9–10, embryos from lot D with young cotyledons which have developed well beyond the fusion stages. × 32.

Sufficient variation in size was found between the youngest and the oldest embryos to make possible such a study, though they were all secured in one collection and dissected out within an interval of only five days, during which the material was kept living, though probably not growing as vigorously as if still attached to the tree. Unfortunately, other collections from the same tree were not possible after an interval of several weeks, for this would very much have simplified the task of studying them by this method. However, the facts were well shown even though based on this one collection of material, because there was considerable difference in size between the youngest and oldest embryos that were obtained.

By studying the embryos in a watchglass with a binocular dissecting microscope, examining them one at a time from all sides, they were divided into four lots. The embryos of lot A included all that were smaller than any of the figures shown; embryos too small to be considered because their primordia had either not appeared or were not distinct enough to be counted with certainty; lot B, embryos like figures 4, 5, 6, and 10, in which there were evidences of either fusing or splitting cotyledons in the primordia stage; lot C, embryos of the same age as B, but showing no evidence of fusion by the grouping of their primordia; lot D, the oldest embryos, all older than B or C, in which, although the cotyledons were not fully developed, they were undoubtedly beyond the critical stages when fusions were found to occur. Figures 7, 8, 21, and 22 are from lot C and figures 9 and 10 from lot D.

The table below summarizes the result of this study. In lot B, all the primordial lobes were counted, even though in some instances they had practically fused with a neighbor, but broad cotyledon primordia which had no double tip were regarded as single primordia.

TABLE I. *The distribution of the cotyledons and cotyledon primordia in Picea mariana.*

Cotyledons or Primordia, Number	Lot B, 23 Embryos, Frequency	Lot C, 83 Embryos, Frequency	Lot D, 63 Embryos, Frequency
3	0	9	5
4	6	53	42
5	18	20	15
6	4	1	1
Total primordia or cotyledons	138	345	264
Average primordia or cotyledons.......	4.93 ± .39	4.16 ± .41	4.17 ± .39
Standard deviation (σ)	.59	.61	.58

Since lot D represents a late stage, and lots B and C represent an early stage, the averages of the number of cotyledons show that there is a reduction in the cotyledon number in the course of their development. Of course, this indicates that the double cotyledon primordia of the embryos in lot B are fusing.

It will be seen that if these were not fusing cotyledons, but in the course of separation, then the oldest group, lot D, might be expected to have an average of about 4.93 cotyledons, the average of lot B, or even more. The assumption that the embryos which were selected for lot C were not undergoing a fusion of their cotyledons was probably correct, since their average number of primordia, 4.16, agrees very closely with 4.17, the average number of cotyledons in lot D. However, lot B had an average of 0.76 cotyledons too many per embryo to agree with the number found in lot D.

A more careful analysis of the embryos of lot B showed that they could be classified in five categories, according to the number of primordia and the manner in which these were appearing to unite in forming cotyledons. On the twenty-eight embryos of the lot, forty-five double primordia were found, which ranged from such cases as were noticeable only when the embryos were held in a certain position, to others in which the fusion was nearly complete and the upper edge of the young cotyledon was only slightly retuse. The diagram of figure 6*a* illustrates the characters of the five general categories, and the figures at the side indicate the number of embryos falling within each group.

It is to be expected that when the appearance of fusion is not very marked, as in many of the instances that were included in lot B, these double primordia will not all fuse to form single broad cotyledons. Many of them will form two distinct cotyledons in spite of the fact that they appeared to be fusing at an early stage.

If we may assume that twenty-eight fusions will occur, an average of one fusion for every embryo in lot B, then the average number of cotyledons produced in lots B and C combined will be 4.10, which is still quite close to the value 4.17, the average of lot D.

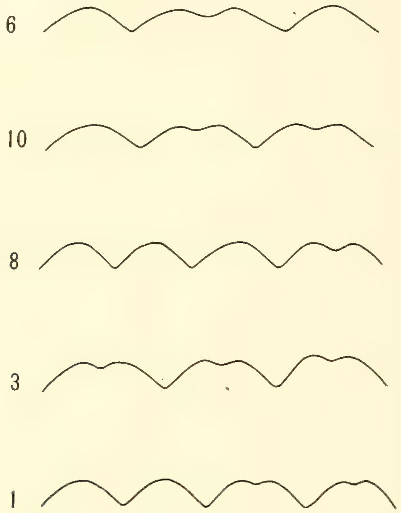


FIG. 6*a*. Diagrams illustrating the character of the five categories into which the embryos of *Picea mariana* (Lot B) were subdivided. The figures to the left indicate the number of embryos in each group.

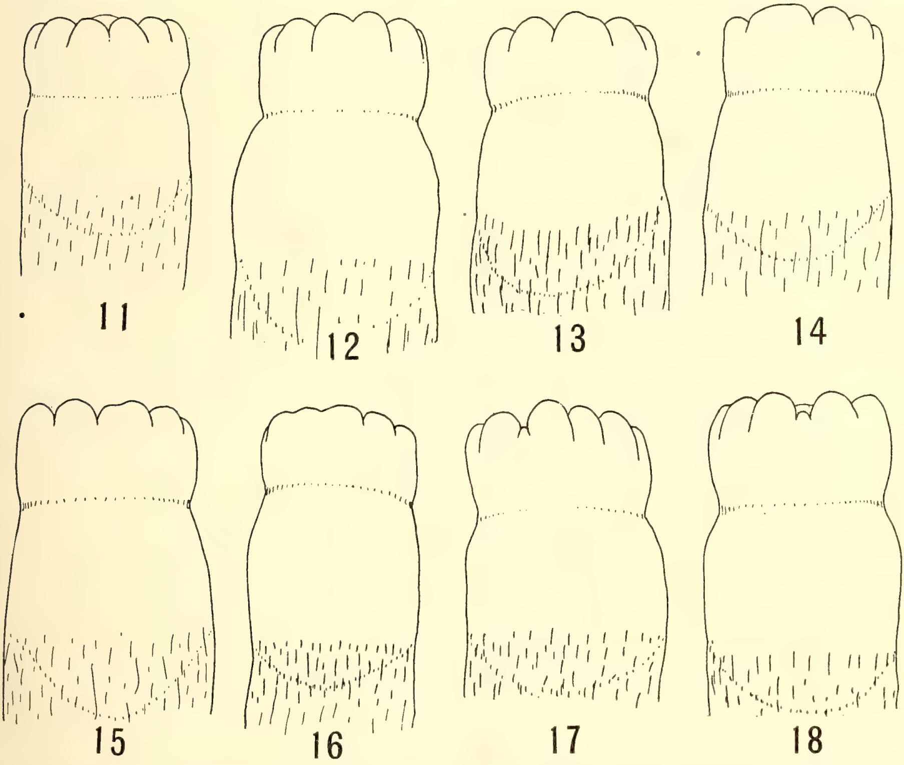
A study of *Larix europaea* gave very similar results. The material was secured at Dundee, Illinois, during the latter part of July 1917. The cones had been poorly pollinated and very few good seeds were found in each cone. The quantity of material was thus quite limited but included material from two collections gathered about a week apart. The embryos are only slightly larger than those of Picea in the cotyledon primordia stage, as is shown in figure 19.

The embryos were separated into lots in the same way as those of Picea, and the results are tabulated below. It is significant that the average number of primordia in lot C agrees quite closely with the cotyledon average found in lot D, and that lot B has in this case an average of 0.55 primordia in excess of the number of cotyledons that it may be expected to produce, based on a comparison with lot D. However, considering the small number of cases that were observed, the conclusion that the cotyledons undergo a fusion in *Larix europaea*, while justified, is by no means so well established as is a similar conclusion in the case of Picea.

TABLE II. *The distribution of the cotyledons and cotyledon primordia in Larix europaea*

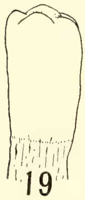
Cotyledons or Primordia, Number	Lot B, 6 Embryos, Frequency	Lot C, 10 Embryos, Frequency	Lot D, 34 Embryos, Frequency
5	1	3	11
6	2	7	20
7	3	0	3
Total primordia or cotyledons	38	57	196
Average primordia or cotyledons	6.33 ± .49	5·70 ± ·31	5·76 ± ·39
Standard deviation (σ)	·74	·46	·59

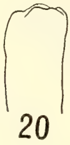
Cedrus is another genus which was found to show fusing cotyledons. Through the kindness of Dr. E. J. Kraus, material of *Cedrus Libani* was sent to Texas from one of the trees growing on the Agricultural College grounds at Corvallis, Oregon. The cones were dissected (July 26, 1918) and were

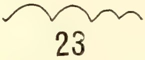


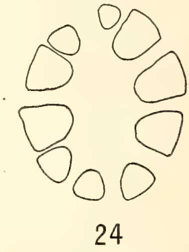
Cedrus Libani. FIG. 11, an ordinary embryo without fusing primordia, or before any fusions may be seen. FIGS. 12, 13, and 15 show stages in the fusion of two cotyledon primordia. FIG. 14 has a broad cotyledon which has no doubt resulted from a fusion of two primordia. FIG. 16 shows three primordia fusing. FIGS. 17 and 18 show reduction of cotyledon number when primordia become aborted. × 32.

found to contain embryos in the cotyledon-forming stages. There was again a great scarcity of good seeds in the cones, a condition which is due to poor pollination and is almost always met with when a species is cultivated out of its native region. That this species should show fusing cotyledons came as a surprise, because there were found to be from seven to twelve cotyledons (average about nine), and the writer had been expecting to find cotyledonary fusions among species with a small number of cotyledons.









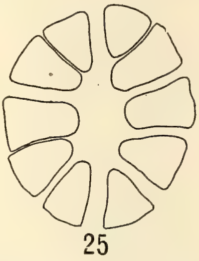


FIG. 19. *Larix* embryo from lot C showing a very slight inequality in the first appearance of the primordia. × 32. FIGS. 20–22, embryos of *Picea mariana* showing a similar slight difference in the origin of the cotyledon primordia. FIG. 20 from lot B, FIGS. 21 and 22 from lot C. × 32. FIG. 23, a diagram illustrating the manner in which cotyledon primordia frequently appear when unequally developed, suggesting that they are essentially spiral.

FIG. 24. Section near the tip of the cotyledons of a fully developed embryo of *Pinus Laricio*, showing a bilateral grouping of the cotyledons. FIG. 25, a similar section taken lower down on the same embryo.

The fusions of the primordia are gradual and so plainly seen that in this case, as in *Pinus Banksiana*, the statistical method is not necessary to convince one of what is taking place. Figure 11 shows an ordinary embryo with cotyledon primordia surrounding the primordium of the stem tip (shaded), and figures 12–16 show a number of typical cases with the fusing cotyledons. Out of twenty-five embryos in cotyledon primordia stages, eight such cotyledons were found which showed that fusions were taking place. Figure 16 shows three primordia that are apparently fusing.

Cedrus has two methods by which its cotyledons are reduced in number. One of these is fusion, and the other is the abortion of primordia as shown in figures 17 and 18. Two instances of this kind were found and are here figured, but in embryos much older than these this vestige would be overlooked, if it could be found at all.

An interesting fact which can be observed in many pine embryos is the tendency to become bilateral. The cotyledons frequently develop in two groups, and this is well shown in figure 24 which is drawn from a cross section near the tip of the cotyledons of an embryo of *Pinus Laricio*. As shown by figure 25, a section taken lower down on the same embryo, this arrangement of the cotyledons in two bilateral groups is noteworthy. It is clear that if wholesale fusions should occur here it would probably result in dicotyledony or in a two-lipped cotyledonary tube. This tendency for the cotyledons to develop in two groups may be found in *Pinus Laricio*, *P. Banksiana*, *P. edule*, *P. Sabiniana*, and doubtless in many other species. It is not usually found in *P. Strobus*.

In *Abies balsamea*, *Juniperus commune*, and *Thuja occidentalis*, no evidences of cotyledonary fusions were found. The cotyledonary number is nearly fixed in these species, being usually four and occasionally five in *Abies balsamea*, and usually two, sometimes three, in Thuja and Juniperus. Not more than several dozen embryos of each of these species were examined in the cotyledon-forming stage, and these all with negative results.

Abies balsamea has a well developed sinus at the tip of each cotyledon, which makes the cotyledon appear strongly retuse. This character is developed after the cotyledons have begun to elongate, and is not due to fusing primordia as one might suspect in the light of the foregoing studies, after examining the cotyledons in any but the earliest stages of their development.

In his work on Pinus, the writer reported that the cotyledon primordia are usually simultaneous in their origin, but exceptions to this were found in which the primordia on one side appeared slightly before those on the opposite side. This tendency has been observed in Pinus, Larix (see fig. 19), Picea (see figs. 20–22), and occasionally in Cedrus, but the difference between the first and last primordia that appear is never very great. The primordia appear so nearly at the same time that on most of the embryos this feature is entirely overlooked. Figure 23 shows diagrammatically this slight difference in the size of the primordia on the same embryo in an extreme case, and this is quite frequently found in *Picea mariana*. It suggests that the cotyledons are essentially spiral in their origin, that they have become cyclic from a previous spiral condition.

Sometimes a zygomorphic tendency becomes evident some time after the primordia have appeared and the cotyledons have begun to elongate. The most extreme case of this kind was reported in a half-grown embryo of *Pinus Laricio* (2), and Picea showed several less marked examples. This zygomorphy which comes on some time after the primordia have appeared is not to be confused with the slight difference in the appearance of the primordia themselves, mentioned above.

The tendency toward spiral development exhibited by these cotyledon primordia suggested the idea that this may have been the more primitive condition, and made it seem desirable to know more definitely the condition of the first leaves of the plumule just above the cotyledons. An examination of the seedlings of several Abietineae shows that though the cotyledons are cyclic, their first simple leaves are arranged spirally, the condition which is well known for the older branches. The following seedlings were inspected: *Abies concolor*, *Pinus Strobus*, *Pinus Laricio*, *Pinus Banksiana*, *Pinus edule, Pinus ponderosa, Pinus Sabiniana, Picea excelsa, Larix europaea* and *Pseudotsuga taxifolia.* It is in the upper axils of these spirally arranged simple leaves that the first needle-bearing branches appear toward the close of the first season's growth, and the older branches have the homologues of these simple leaves in the form of scales that likewise subtend the spur shoots.

Discussion

The cotyledon primordia frequently fuse in *Pinus Banksiana* and *Cedrus Libani,* and this fact is so evident that it is not open to question, for here the process of fusion, when it occurs, is prolonged into the early stages of cotyledon elongation. The situation in Picea and Larix is not so simple, because the fusions of the primordia occur more suddenly, before the cotyledons elongate, making it necessary to employ the statistical method.

The appearance of a larger number of primordia than cotyledons is apparently a recapitulation of a feature in the phylogeny of the conifers, and since the examples chosen for study were selected from several different genera of the Abietineae, it indicates that the fusion tendency is quite general in this group. Occasionally the process expresses itself in other ways than by fusions, for a primordium sometimes becomes aborted in *Cedrus Libani.* It should be remembered that though some species failed to show fusions, no ontogenetic evidence of a splitting of cotyledons has been found in any species.

Recapitulation in external anatomy has long been recognized in the seedling stages of the conifers, such as Thuja, Phyllocladus, Ginkgo, etc. Jeffrey, in his recent work (8), cites numerous internal anatomical features which show recapitulation in the seedling stages. It is, therefore, not surprising that recapitulation should be found in the stages of the embryo before the seeds are shed. When the embryos are still enclosed within the seeds they are affected far less by the external conditions which bring about diversity. The writer has also demonstrated instances of recapitulation in the early embryos of the pine by finding that an apical cell of the pteridophyte type persists until an embryo mass of several hundred cells has been formed. Recapitulation has therefore been found in stages earlier as well as later than this cotyledon stage, and it is only to be expected that the cotyledon primordia should be found to show well marked tendencies to fuse if this is the manner in which dicotyledony has arisen.

The number of cotyledons in many species of gymnosperms is quite variable, and this variation has had a tendency to obscure any evidences of recapitulation. A fact which is significant in this connection is the well recognized tendency for a primitive or genetic form to show varying characters. A familiar illustration of this is shown in the number of petals, stamens, or carpels found in the Ranunculaceae, which belong to one of the lowest orders of the Archichlamydeae. Here the flower parts are spiral or indefinite, and even when they become cyclic in the Ranales they are subject to fluctuation. It is easy to find a buttercup with extra petals, but it is seldom that the flower of a Phlox or a bluebell will show variations from the regular floral formula. Therefore it is very reasonable to regard this variation in the cotyledon number as an earmark of the low genetic position of the pines among the Coniferales.

This view falls in line with recent paleobotanical investigations which show that Pinus and the earlier form Prepinus are historically the most ancient conifers, also with the primitive position assigned to Pinus by the writer on the basis of its embryogeny.

Again, turning to our comparison of the cotyledons with floral structures, it is noteworthy that when floral members are spiral, their number is subject to the widest variation. Cyclic parts do not fluctuate so much, but when the cyclic condition is only very recently established they are also subject to considerable variation. Similarly, if the cotyledons still retain traits that characterize the spiral arrangement, this would account for the variation in their number which may be found in different individuals of any species. The first simple leaves that appear above the cotyledons in the young seedlings are spiral, and this also suggests that the cotyledons, which are doubtless modified from the first of these simple leaves, were originally spiral but have become more or less cyclic. The appearance of primordia on one side of the embryo before they are visible on the other is therefore also a vestigial character and an evidence of the original spiral condition of the cotyledons. Figure 23 shows the condition of the cotyledon primordia of many embryos, which may be taken to suggest that they are essentially spiral in their origin. These three features, namely, the variation in the cotyledon number, the spiral arrangement of the first leaves, and the slight tendency for the cotyledon primordia to appear on one side earlier than on the other, constitute the basis for the conclusion that the cotyledons themselves have become cyclic from a primitive spiral condition. To this might perhaps be added the evidence from the occasional displaced plumular leaves which Hill and DeFraine find added to the cotyledonary node.

It is further interesting to call attention to the close parallel which seems to exist between the evolution of the floral members in angiosperms and that of the cotyledons of the embryo. In the flower, we pass from indefinite polypetaly to definite numbers in the floral members, then to sympetaly, which results in the corolla tube; this becomes two-lipped and finally ligulate, aside from its many other variations. A similar evolution has probably taken place in the history of the cotyledons. Originally, the cotyledons may have been spiral, but when the seed habit became established they soon became cyclic, the polycotyledonous condition today; these reduced their number by fusions, or occasionally by such methods as abortion of primordia. In some forms fusions became more general, resulting in the cotyledonary tubes.

Dicotyledony may have been attained in more than one way. One of these would be by checking the growth of the cotyledonary tube in two places during its development, much as a strongly bilabiate corolla develops. Another method is by a fusion of the cotyledons in two groups. This is strongly suggested by the bilateral grouping of the cotyledons in *Pinus Laricio*, and doubtless the tendency for the cotyledons to fuse, acting in concert with the cause which brings about this bilateral symmetry, could produce dicotyledony from polycotyledony.

That cotyledonary tubes are frequently found among gymnosperms was brought out by the work of Hill and DeFraine. The recent work of Hutchinson (7) on Keteleeria also shows that this embryo has a well developed cotyledonary tube and at the same time only four cotyledons, a rather reduced number. The fact that cotyledonary tubes have been found in angiosperms connects these with the polycotyledonous gymnosperms. Coulter and Land (4) have shown in a recent investigation how monocotyledony has been derived from dicotyledony by a zygomorphic development of the cotyledonary zone of an embryo which has a cotyledonary tube in an early stage of its development.

The writer has expressed the opinion that the cotyledonary tube had its origin in cotyledonary fusions. This is further suggested by the fact that Agapanthus has two primordia on a cotyledonary tube while Cyrtanthus has four primordia (4), and no doubt these are still further examples of embryonic recapitulation of an ancestral character, therefore distinctly pointing to polycotyledony as the more primitive condition.

The account which Coulter (3) gives for grass embryos as well as the one for Cyrtanthus completes our analogy between embryo and corolla development, for these monocotyledonous embryos come to correspond to the unilabiate or ligulate corolla by becoming extremely zygomorphic.

Summary and Conclusions

The results of this investigation show that in a number of conifers fusions of the cotyledons occur during their embryonic development. It is significant that no evidences of splitting cotyledons were found in any species.

The larger number of primordia found in the species exhibiting fusion is a recapitulation of a more primitive condition in which a larger number of cotyledons existed.

The fusion of cotyledons has given rise to a reduced number of cotyledons and also to cotyledonary tubes in some species. The occurrence of cotyledonary tubes in gymnosperms and the retention of this feature in some angiosperm embryos points to polycotyledony as the primitive condition.

All the definitely known facts brought out by others, as well as the investigations of the writer, may be used to support the following conclusions:

The primitive gymnosperm embryo had numerous cotyledons, which were imperfectly cyclic and variable in number. These cyclic cotyledons were in all probability derived from spirally arranged leaves that became cyclic in the cotyledonary node. Cotyledonary fusions reduced the mumber of cotyledons and also produced cotyledonary tubes in many species. Dicotyledony was attained either by a general fusion of many cotyledons in two groups, or by an extremely bilabiate development of a cotyledonary tube; and monocotyledony is the result of a cotyledonary tube becoming unilabiate in the course of its development. The polycotyledonous condition is therefore primitive and the dicotyledonous one is derived.

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THE RÔLE OF SEDGES IN SOME COLORADO PLANT COMMUNITIES

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Every botanist is well aware of the large part taken by sedges in the vegetation of lake shores and swampy areas. For the eastern United States the species involved are well known, as also their relative importance in different stages of succession. Sedges of lake shores in the Rocky Mountains have been referred to by Clements (1), Ramaley and Robbins (7), Ramaley (4), and Robbins (9). In the present paper additional facts are given as to sedges of pondsides together with a series of observations on sedges of mesophytic and xerophytic habitats. This paper is based upon studies carried on largely from the University of Colorado Mountain Laboratory at Tolland, Colorado. Statements in the paper apply chiefly to the northern part of the state and to the mountain districts rather than to the plains. All species mentioned are well-known constituents of the Colorado flora. The nomenclature employed is that of Rydberg's "Flora of the Rocky Mountains and the Adjacent Plains" (New York, 1917). The various life zones are given the following names in accordance with customary usage: Plains, Foothill, Montane, Subalpine, Alpine (4).

Sedges exist chiefly in primitive communities or unstable situations. An area in a region that is climatically mesophytic becomes eventually either forest or grassland in which sedges have a very small place. So also, a climatically xerophytic area grows poor in sedges as the ultimate type of vegetation appears. Most sedges are aquatics or marsh plants or else they are xerophytes. Only a few are true mesophytes, and even these are likely to become crowded out by grasses and herbaceous dicotyledons in a meadow which has developed from marsh. Meadow or prairie of xerarch origin is also typically without sedges, the xerophytic sedges of more primitive stages disappearing before the meadow stage is reached. In the following systematic account the various genera of Cyperaceae are briefly considered, but chief attention is given to Carex.

SCIRPUS

Scirpus lacustris and other species of the genus are too well known everywhere to need description or comment. At lower elevations in Colorado, *i. e.*, on the plains, they behave very much as in the eastern part of the United States. They are found to some extent in the foothill area but are typically absent from lakes of the montane and higher zones.

ELEOCHARIS

Eleocharis acicularis, a small tufted spike-rush, is to be found somewhere along the shore of almost every lake or pond in the plains, foothill, and montane regions of Colorado. It does not grow on a coarse-grained substratum, but becomes established in fine sand, in clay, or in loam. Being firmly fixed by rhizomes and fibrous roots and producing a dense matted growth, it is a good soil binder against wave action. In Boulder Park, at Tolland, Colorado, as shown by Robbins (9), *Eleocharis acicularis* is associated with *Ranunculus reptans* in forming the characteristic pioneer community of mud flats along streams.

Eleocharis palustris, a much larger species, grows more in the water and often in almost pure stand, effectively filling up shallow pools in a few years. Both spike-rushes occur all the way from the plains to subalpine stations. A great many montane and subalpine lakes are, however, entirely free from *Eleocharis lacustris.* Its place is sometimes taken, ecologically, by *Sparganium angustifolium.* Other species of Eleocharis occur in the area studied, but the writer has slight acquaintance with them.

CYPERUS

Although five species of Cyperus are reported from Colorado, not any of them are conspicuous or important. They are confined to the plains region where they occur along streams and at margins of lakes or reservoirs, usually in wet, sandy soil. The commonest species is *Cyperus inflexus*, a rather diminutive annual confined to sandy shores recently exposed by lowering of a lake level or shifting of a stream bed. Such habitats are likely to be occupied soon, if not in the first place, by *Agrostis hiemalis*, *Eleocharis acicularis*, or *Alopecurus aristulatus.*

ERIOPHORUM

The species of Eriophorum, three in Colorado, are confined to habitats where the substratum is of a peaty nature. The writer has seen them only as very minor members in the Montane-Subalpine Sedge Moor Association.

DULICHIUM, FIMBRYSTILIS, ELYNA

These genera, represented in Colorado by one, two, and one species respectively, are of rare or unusual occurrence. The writer has made no observations of interest upon them.

CAREX

Carex is well represented in northern Colorado. Unlike other genera of Cyperaceae which are practically all marsh plants, there are many species of Carex in mesophytic and xerophytic situations. In the following account the various association types in which Carices are important, together with the associations which belong to them, are briefly indicated. The categories, *association*, *association type*, *society*, *climatic climax association*, etc., are employed as defined by Nichols (3).

List of Plant Communities in which the Rôle of Carex is Considered

1. Half-submersed Carex Association Type (Inceptive Sedge Moor).
    - (*a*) Half-submersed Carex Association of moderate altitudes.
    - (*b*) Subalpine Half-submersed Carex Association.
2. Sedge Moor Association Type.
    - (*a*) Plains-Foothill Sedge Moor Association.
    - (*b*) Montane-Subalpine Sedge Moor Association.
    - (*c*) Alpine Sedge Moor Association.
    - (*d*) Snow Patch Sedge Association.
3. Meadow Association Type.
    - (*a*) Plains-Foothill Streambank Meadow Association.
    - (*b*) Prairie Grass Association (of mesas and foothills).
    - (*c*) Montane Meadow Association.
    - (*d*) Subalpine Meadow Association.
    - (*e*) Alpine Meadow Association.
4. Xerophytic Carex Grassland Association Type.
    - (*a*) *Carex stenophylla* Grassland Association.
    - (*b*) *Carex rossii* Grassland Association.
    - (*c*) *Carex siccata* Grassland Association.
    - (*d*) *Carex elynoides* Grassland Association.

Half-submersed Carex Association Type (Inceptive Sedge Moor).

A number of species of Carex occur commonly in water at the margins of ponds or lakes or meandering streams. In such situations they are pioneer soil formers, as they lessen wind and wave action and favor the accumulation of humus. These same species often occur in sedge moor and will therefore be listed also in the account of that association type.

(*a*) *The Half-submersed Carex Association of moderate altitudes.*—Here the species are chiefly *Carex rostrata*, *C. lanuginosa*, *C. vesicaria*, *C. canescens*. In addition to these, almost any of the sedge-moor Carices may be found locally in standing water. In montane lakes *C. rostrata* is the commonest species.

(*b*) *The Subalpine Half-submersed Carex Association* consists chiefly of *C. aquatilis*. Except in lakes that are already considerably insilted, it does not form a complete circum-area but occurs near the inlet or outlet.

There is no half-submersed zone in alpine lakes. This is due partly to the fact that they are geologically young, the lake bottom being made of large broken rocks with no soil. Another reason is that these lakes are frozen over from November to June, or as late as July. Even during August, the water temperature as shown by Dodds (2) is low, about 10 degrees Centigrade. Wherever the vegetation comes close to the edge of the water it is a dense growth of mosses and sedges, *i. e.*, a *moss moor*. This is usually elevated as a distinct "rim" one or two decimeters higher than the sedge moor, just as pointed out for montane lakes by Robbins (9). *Carex scopulorum* is probably the commonest sedge of the "rim."

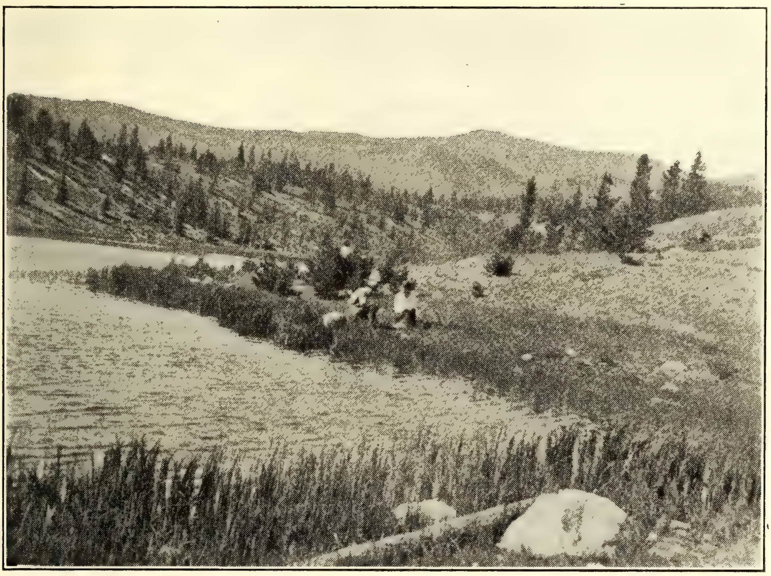

FIG. 1. A high montane lake (Teller Lake) showing Half-submersed Carex Association of *Carex rostrata* and *Carex canescens*. This is followed by a narrow zone of Sedge Moor dominated by *Carex aquatilis*. Outside of this is a strip of Meadow in which *Carex festivella* is important; farther out is a mixed Dry Grassland with a large amount of *Carex siccata*.

Sedge Moor Association Type

This type of plant community exists under conditions similar to that described in the preceding section, but the soil is not covered by water. Ponds and lakes occur abundantly in the montane, subalpine, and alpine zones, and they always have some bordering sedge moor. The lakes at higher elevations are either morainic or of the rock-basin type. In the montane parks meandering streams give rise to ox-bows. The foothill area with its older topography and better drainage has very few lakes. A considerable number of natural lakes exist on the plains. Some of these are of ox-bow origin, some occupy old channels closed by alluvial damming, and some basins have arisen through wind action upon weathered sand-stone or shale. All of these various bodies of water afford stations for sedges. The many artificial reservoirs for irrigation show a scant shore vegetation because of the frequent great changes in water level.

The term *sedge moor* as used by the present writer is meant to include all communities in wet soil dominated by Carices and often having a considerable amount of moss.[1] In some places it dries out to a degree in late August, but is saturated or nearly saturated during most of the growing season. According to Robbins (9), the soil water has a slightly acid reaction.

Sedge moor is an early stage in the hydrarch sequence. As the soil becomes built up through peat formation, or as the related stream or lake is lowered so that the water table sinks farther below the surface, a willow thicket develops or else a wet meadow. If it is the willow thicket that is produced, it will, in turn, be followed by meadow, and this again, in mountain districts, may be replaced by coniferous forest.

In Colorado, as pointed out by Robbins (9), Sphagnum bog does not occur. The nearest approach to it is sedge moor. It is true that Sphagnum moss is found locally in montane and subalpine situations, but it is not abundant. Most of the characteristic plants of bogs of the eastern United States are not present in Colorado at all; a few exist in isolated localities.

In all sedge moors Carices cover from 60 to 90 percent of the soil surface. Mosses are abundant, and liverworts (Marchantia) also, except at the higher elevations. Grasses are generally present, differing in species with the life zone; and a number of dicotyledons occur, chiefly of the Polygonaceae, Alsinaceae, Ranunculaceae, Gentianaceae, Scrophulariaceae, Carduaceae, and Cichoriaceae. As would be expected from likeness of edaphic conditions, there is considerable floristic similarity in sedge moors, even between those rather widely separated in altitude.

Three sedge moor associations may be recognized in northern Colorado, named in accordance with the life zones in which they commonly occur. The species, so far as known to the present writer, are listed for each association,—roughly in order of importance.

(*a*) *Plains-Foothill Sedge Moor Association: Carex lanuginosa, C. nebraskensis, C. aquatilis, C. rostrata, C. canescens, C. stipata, C. lasiocarpa, C. vesicaria, C. tenuirostris.*

(*b*) *Montane-Subalpine Sedge Moor Association: Carex aquatilis* (characteristic).—There are two societies. The *Montane Society* has the following secondary species: *Carex rostrata, C. vesicaria, C. lanuginosa, C. canescens, C. halleri, C. illota, C. lasiocarpa, C. tenuirostris, C. disperma, C.*

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[1] Along streams the proportion of moss is often very high and the association becomes so modified as to be more properly designated as *moss moor*. The same term may be applied to that part of the moor of certain lakes which is closest to the water. High-altitude lakes show often other types of moor also, *viz.*: a *meadow moor* and a *heath moor*.

aurea, C. paupercula. In the *Subalpine Society* there are subsidiary species as follows: *Carex scopulorum, C. nigricans, C. paupercula, C. phaeocephala, C. illota, C. albonigra.*

(*c*) *Alpine Sedge Moor Association: Carex scopulorum, C. nigricans, C. nelsonii, C. chalciolepis, C. capillaris.—Carex scopulorum* and *C. nigricans* are common around springs and in seepage areas everywhere near the top of the Continental Divide.

(*d*) *Snow-Patch Sedge Association.*—In the subalpine and alpine zones snow drifts often remain on the ground until midsummer. In such places, if there is some accumulation of fine-grained soil, *Carex nigricans* is likely to form an almost pure stand. The snow accumulates year after year at the same point, and the very short period each year in which the ground is free from snow does not permit the establishment of many plant species. A typical area of snow-patch sedge association is made up almost wholly of *Carex nigricans.* A few subordinate species are represented by scattered individuals in very small amount. These are *Carex scopulorum, Ranunculus adoneus, Sibbaldia procumbens, Caltha rotundifolia, Trollius albiflorus,* Polytrichum and other mosses. The general appearance is quite different from that of ordinary sedge moor because of the low stature and broad leaves, widely spreading, of the dominant species. The snow-patch sedge association is interesting as being a rather primitive community and at the same time an ultimate one. The edaphic conditions are such that no successional stage can develop, at least so long as the mountains stand and snow continues to fall and to drift each winter.

Meadow Association Type

Meadow, as here understood, comprises all mesophytic grassland, but does not include anything that could be called sedge moor or marsh. Meadow grassland at lower altitudes in Colorado occurs in rather small strips where edaphic conditions are favorable to its development. The plains and foothill regions are too dry to support meadow on level ground or on south-facing slopes. Alluvial fans of fine-grained black soil support a mesophytic grassland designated by Vestal (10) as the Western Mesophytic Prairie Grass Association. Fringes of meadow occur along water courses, especially at bends of streams, and also next to (*i.e.*, in drier soil than) the sedge moor or willow thicket of mountain lakes. Hillside meadows, sometimes of considerable extent, occur in the montane zone. The higher rainfall and lower temperature of the subalpine and alpine zones are suitable for meadow development, and the association is often well developed. Lack of humus in the soil is, however, a limiting factor of importance.

A number of species of Carex occur in meadows, but only a few are abundant. The most important one is *Carex festivella.* It extends through foothill, montane, and subalpine zones and to the lower part of the alpine. In the montane zone, where it is most abundant, it is associated with the plants named later as characteristic of the Montane Meadow Association. *Carex ebenea* has much the same distribution. These species sometimes dominate rather definite societies. As the soil becomes better drained the Carices are likely to be crowded out by grasses and dicotyledons. They are to be considered then as belonging to edaphic rather than to climatic meadows.

Carices of meadows and other mesophytic situations in addition to those thus far mentioned are all of less importance. The following are known to the writer: *Carex praegracilis*, *C. pachystachys*, *C. praticola* of moderate elevations, and *C. albonigra*, *C. bella*, *C. illota*, and *C. nova* in the subalpine and alpine zones. *C. chalciolepis* occurs in moist parts of alpine meadow. *C. douglassii* is a dry grassland species which is found to some extent in foothill meadow, while *C. siccata*, a species of dry hillsides and forest openings, is sometimes locally frequent in meadows of the montane zone.

The more typical meadows of northern Colorado may be distinguished as follows:

(*a*) *Plains-Foothill Streambank Meadow Association*, originally dominated by *Poa pratensis* but now much modified by the presence of *Trifolium repens* and *Phleum pratense*. This type of meadow is commonly without Carex.

(*b*) *Prairie Grass Association*, of Vestal (10, 11), belonging to lower alluvial slopes of mesas and foothills. Carices are of slight importance. *Carex heliophila* may be locally abundant in drier parts which have not yet reached the true meadow stage. In moister situations, especially where the soil has been recently carried in and the meadow is still in an inceptive stage, *Carex festivella*, *C. praegracilis*, and others may be present; their stay is quite temporary.

(*c*) *Montane Meadow Association*, of streambanks and pondsides, highly variable in floristic composition and with many consociations and societies as shown by Reed (8); often including *Potentilla pulcherrima*, *Erigeron macranthus*, *Fragaria glauca*, *Valeriana edulis*, *Pentstemon procerus*, *Tium alpinum*, *Pedicularis parryi*. Here there is often a considerable amount of Carex, especially *C. festivella* and *C. ebenea*.

(*d*) *Subalpine Meadow Association*, with such principal species as *Erigeron salsuginosus*, *Potentilla diversifolia*, *Antennaria umbrinella*, *Ligusticum tenuifolium*, *Castilleja lauta*, and *Castilleja rhexifolia*. In this association also the Carices are *C. festivella* and *C. ebenea*, with a sprinkling of various relicts of the former sedge moor stage.

(*e*) *Alpine Meadow Association*, with the following as some of the principal species: *Acomastylis turbinata*, *Bistorta bistortoides*, *Castilleja occidentalis*, *Trifolium dasyphyllum* and *Trifolium parryi*, *Rydbergia grandiflora*, *Campanula petiolata*. Associated Carices are quite frequent and may be almost any of the species previously noted as belonging to high altitudes. Even some of the sedge-moor Carices may here enter the meadow, since the limits of plant associations in the alpine zone are not at all clearly marked.

Xerophytic Carex Grassland Association Type

Four xerophytic Carices are especially important as being the dominant species of definite associations, often covering areas of considerable extent. These associations will be considered in order, beginning at the lower altitudes.

(*a*) *Carex stenophylla Grassland Association.*—This is a highly primitive association, well developed in open parks of the foothill and montane zones in coarse sandy and gravelly soil lacking in humus. Plants of *Carex stenophylla* are low, generally less than 1 dm. in height. They spread by means of rhizomes and produce a rather thin sod. In dry grassland of a mountain park at Tolland, Colorado (altitude 8,889 feet), as previously reported by the present writer (5), this association is well represented. Associated plants are such pioneers as *Selaginella densa*, *Erigeron trifidus*, *Potentilla concinna*, *Potentilla strigosa*. As soil conditions become more favorable through accumulation of humus the Carex becomes less important, its place being taken by such grasses as Festuca, Agropyron, Koeleria, Danthonia, and Muhlenbergia. The *Carex stenophylla* association is, then, the inceptive stage of typical xerophytic grassland dominated by true grasses.

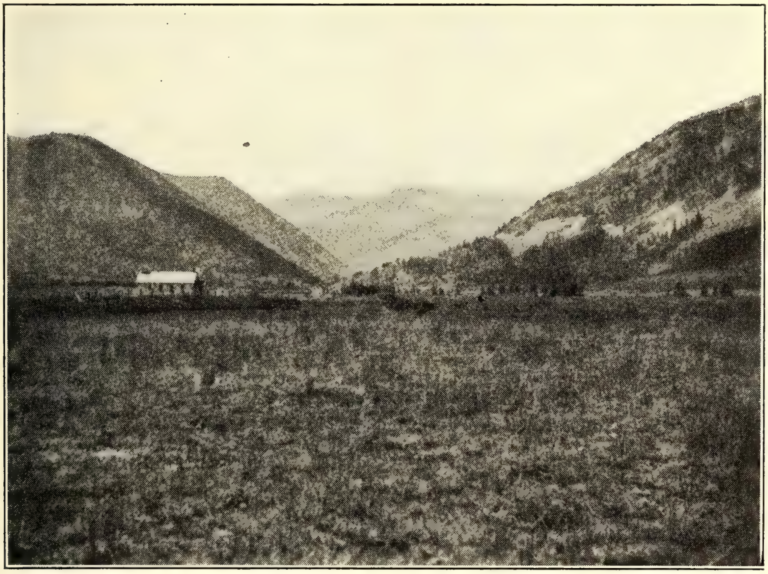

FIG. 2. *Carex stenophylla* Association on an old flood plain at Tolland, Colorado. The Carex plants, scarcely a decimeter tall, form a thin sod. At this particular point very few plants of secondary species are present.

(*b*) *Carex rossii Grassland Association.*—This community appears in clearings and burned areas of coniferous forest on exposed hillsides of the montane and subalpine zones. The plants of *Carex rossii* form dense mats from 1 dm. to 1 m. across, developing in sandy or gravelly soil especially on south and west exposures. Subordinate plants of the *Carex rossii* grassland are *Vaccinium caespitosum*, *Thermopsis divaricarpa*, *Chamaenerion spicatum*, *Carex siccata*, *Rubus melanolasius*, and various forest plants in small numbers. There are some lichens on the ground, especially species of Cladonia and Peltigera under stones or old logs. This association may give way soon to coniferous forest, since seedlings of *Pinus flexilis* and *Pinus murrayana* are likely to develop. When the new forest is well started the Carex has all but disappeared, it being intolerant of shade. In some situations, however, the reproduction of pines is poor, and it may be many decades or even centuries before the Carex grassland gives way to forest.

(*c*) *Carex siccata Grassland Association.*—This and the preceding community might be classed by some students as consociations, but to the writer they seem so different as to demand associational standing. *Carex siccata* spreads by rhizomes and forms a loose sod; it does not produce dense mats at all. *Carex siccata* is more likely to appear at higher altitudes. It is more tolerant of shade and less xerophytic than *C. rossii.* The associated plants with *C. siccata* are those already mentioned for the *C. rossii* grassland with some additions such as *Tessaranthium stenopetalum*, *Koeleria gracilis*, *Amarella plebeja*, Sambucus (scattered), etc. The *Carex siccata* grassland is often a temporary climax having a long period of existence. Frequently occurring near timberline where the establishment of tree seedlings is difficult at best, the Carex occupies the soil and may maintain itself for centuries without invasion.

(*d*) *Carex elynoides Grassland Association.*—This community is found on mountain tops and slopes in the alpine zone or occasionally at lower elevations. Unlike the two associations just named, this one does not develop so often on steep slopes with poor soil but on more nearly level ground where humus may accumulate. It is not, however, to be considered as mesophytic, differing markedly as it does from the hydrarch alpine meadow. Soil is drier and the vegetation cover is not so close. *Carex elynoides* is a densely cespitose species and will be best understood if described as the alpine form of *C. filifolia.* It occupies from 40 to 80 percent of the soil surface. Associated plants are partly xerophytes, as *Selaginella densa*, *Silene acaulis*, *Oreoxis alpina*, *Festuca minutiflora*, and *Tetraneuris lanigera*, and partly mesophytes, as *Trifolium dasyphyllum*, *Acomastylis turbinata*, *Rydbergia grandiflora*, and *Castilleja occidentalis.* This mingling of xerophytic and mesophytic forms is common everywhere in high altitudes because of the great diversity of soil depth and soil moisture in even a small area, presence of large rocks, and other disturbing factors. *Carex elynoides* grassland will probably become in time alpine meadow, but perhaps it will be somewhat different from the present hydrarch meadow, the characters of which depend largely on local edaphic conditions. Just what the climatic alpine meadow will be toward which both the xerarch and hydrarch series necessarily tend is not now quite apparent. For a long time to come the *Carex elynoides* grassland will be found just where it now exists. It is of a more permanent character, because more mesophytic, than the communities dominated by *Carex stenophylla*, *C. rossii*, and *C. siccata*.

Carex in Various Xerophytic Associations

Less important xerophytic Carices may be listed without extended comment. *C. heliophila* (*pennsylvanica* of western authors) is frequent in the mixed grassland of mesas and foothills, extending occasionally to the montane zone. Other species found at moderate elevations, none of them of common occurrence, are *Carex brevior*, *C. occidentalis*, *C. xerantica*, *C. douglasii*. In the montane zone *Carex oreocharis* is somewhat abundant and *C. obtusata* rather occasional in dry grassland of parks; *C. geyeri* occurs in dry forest openings. At high altitudes *Carex phaeocephala* and *C. pyrenaica* are frequent species of dry rocky slopes. *C. perglobosa* is occasional on alpine ridges.

List of Species of Carex[2]

In the following list the species of Carex are arranged in three groups according to the "soil moisture index" employed by the writer (6). It need hardly be stated that species may sometimes be found outside their accustomed habitats.

(*a*) *Species in water or wet soil* (marsh plants), the soil moisture index 8, 9, or 10: *aquatilis, aurea, canescens, capillaris, chalciolepis, disperma, halleri, illota, lanuginosa, lasiocarpa, nebraskensis, nelsonii, nigricans, paupercula, phaeocephala, rostrata, scopulorum, stipata, tenuirostris, vesicaria.*

(*b*) *Species in meadow or other mesophytic situations*, the soil moisture index 6 to 7 or sometimes 5: *albonigra, bella, ebenea, festivella, illota, nova, pachystachya, praegracilis, praticola.*

(*c*) *Species in xerophytic situations*, the soil moisture index typically 4 but varying to 5 or 3: *brevior, douglasii, elynoides, geyeri, heliophila, obtusata, occidentalis, oreocharis, perglobosa, phaeocephala, pyrenaica, rossii, siccata, stenophylla, xerantica.*

SUMMARY

The foregoing paper deals with the part played by sedges in the plant communities of northern Colorado. It is based upon studies in all the life zones from plains to alpine heights. The various genera of Cyperaceae are considered in order, but chief attention is given to Carex. Brief statement is made of the association types in which Carices are prominent. The several associations belonging to these types are characterized as to ecological relations and floristic composition. Some of the subject headings are: Half Submersed Carex Association Type, Sedge Moor Association Type, Snow-Patch Association, Meadow Association Type, Xerophytic Carex Grassland Association Type.

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[2] This is not a complete list of the Carices of northern Colorado but includes only the species mentioned in this paper. Most of the author's collections were originally identified by Dr. Theodor Holm, but recently the specimens have been gone over again by Kenneth K. Mackenzie, Esq. Thanks are due to both these gentlemen for their courtesy and painstaking care.

It is pointed out that most sedges belong to early stages of succession in the vegetation of a region, some being prominent in the hydrarch and some in the xerarch sequence. As mesophytism is approached from either direction other species may become prominent for a time, but these are displaced by grasses and dicotyledons in the ultimate climatic association.

A list is given of 44 species of Carex, of which 20 are classed as water and marsh plants, 9 as species of meadow or other mesophytic situation, 15 as species of xerophytic habitats.

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BOTANICAL SOCIETY OF AMERICA

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PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
At 41 North Queen Street, Lancaster, Pa.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879

AMERICAN JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

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BOTANICAL SOCIETY OF AMERICA

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The pages of the Journal are open to members of the Botanical Society of America, or candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

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AMERICAN
JOURNAL OF BOTANY

VOL. VI APRIL, 1919 NO. 4

THE INFLUENCE OF LIGHT UPON THE ACTION OF STOMATA AND ITS RELATION TO THE TRANSPIRATION OF CERTAIN GRAINS

JOHN GRAY AND GEORGE J. PEIRCE

The stomata of leaves are the passages through which the necessary exchange of gases takes place between the interior of the land plant and its leaves and the outside world. They are the organs through which CO_2 and O_2 are taken from the air and given back to it. And it is through them that water vapor passes out into the air. The stomata are the exterior openings of intercellular spaces which may extend for great distances in the body of the plant. These openings are bounded by paired guard cells possessing in most plants a markedly greater degree of elasticity than the other epidermal cells. The mechanism and the behavior of stomata have been the object of study of many botanists for decades. For the older literature of the subject one may refer to Pfeffer (1). Only the few more recent papers bearing on the specific question which we asked ourselves will be referred to here.

In general the behavior of the stomata of the Gramineae is as follows. When the opposite ends of the guard cells of a pair are in contact with each other the stoma is thereby closed. These opposed ends are thin-walled, the rest of the wall being thick and stiff. Opening begins with the expansion of the guard cells. This results in the separation of the guard cells, at first at the ends of the stoma, and later in the middle as well, the guard cells straightening and giving to the pore its familiar oblong shape and its uniform width for nearly its whole extent. Closing appears to be the reverse of this process. This action of the guard cells seems to be a mechanical process dependent upon the character of the environment in which the plant lives.

The times, conditions, and significance of the opening and closing of stomata have interested many plant physiologists, and there are certain general conceptions current, as indicated by the concise statements of the textbooks. But the work of one of us in the field, in which the possible effect of sulphur dioxide fumes upon the stomata, as well as upon the other cells of the leaf, became a matter of importance, made it necessary to ascer-

[The **Journal** for March (6: 87–130) was issued March 28, 1919.]

tain the conditions of opening and closing and the connection of these phenomena with transpiration, a process the importance of which is inversely proportioned to the amounts of moisture in soil and air. Thus in the far west, where "dry farming" is resorted to, it is of the utmost importance to the plant to be able to control its transpiration; and, at the times of most rapid evaporation from all wet masses, to be able to hold its water in spite of the temperature and dryness of the air. It is obvious that water vapor will escape more rapidly along a continuous volume of air, for example through an open stoma, than across one or more membranes, no matter how permeable, interposed between two volumes of air of different humidities, areas and other things being equal. What then causes the opening and closing of stomata? And what are the consequences? On these questions the studies of Darwin and Lloyd bear directly.

Darwin (2), working under the well known climatic conditions of Cambridge, England, which are the general conditions in which the plant physiology of today has been developed, and Lloyd (3), experimenting at the Desert Botanical Laboratory of the Carnegie Institution, at Tucson, Arizona, with climatic conditions not altogether easy for the plant physiologist of other so-called temperate climates to grasp, came to conclusions sufficiently divergent to justify us in reëxamining the subject, especially as we live in a region the climatic conditions of which are near the mean between these two. Darwin, studying the leaves of ivy (*Hedera helix* L.) and of laurel (*Prunus laurocerasus* L.), claims that transpiration is regulated mainly by the stomatal aperture. His results on individual leaves do not clearly show this; but examining the series as a whole, it is seen that the transpiration and the condition of the stomata appear to be related, usually with the relative transpiration somewhat exceeding the stomatal ratio. Lloyd worked on the ocotillo (*Fouquieria splendens* Engelm.) and a showy verbena (*Verbena ciliata* Benth.), both desert plants growing near his laboratory. The study of these extreme types led him to conclude that the "regulatory function of the stomata is almost *nil*." During the day the outgo of water in the ocotillo is greater than the intake. The reverse is true at night. The stomata are open during the day, in which time also there is the greatest decrease in leaf weight. At no time is this effectively regulated by the movements of the guard cells. The stomata do not appear, therefore, to afford the plant any protection against evaporation during the times when evaporation is most dangerous.

The opening and closing of the stomata have been attributed to the changes in the turgidity of the guard cells, their particular shape and their relations to the adjoining epidermal cells causing the changes in turgor to open and close the stomata. Von Mohl (4) and Schwendener (5) were the first to demonstrate this. The latter showed that the convex walls of the two guard cells, facing each other, are more extensible than the flat or concave walls adjoining the next cells of the epidermis. Increasing turgidity stretches the guard cells, their walls flatten or remain unchanged according to their thickness, and the slit between the two opens and widens. Decreasing turgidity has the opposite effect. Thus the direct connection between turgor changes and stomatal movements has been generally accepted, and Darwin and Loyd seem to assume, in their investigations, that this is the case.

Our study of these papers has led us to make certain experiments which cause us to conclude *first*, that the turgidity of the guard cells is a necessary factor in producing and maintaining their elasticity, but *second*, that the direct and indispensable agent in controlling the opening and closing of the stomata is sunlight, which acts as a stimulus upon the guard cells themselves. Their peculiar form and position give them a freedom of movement, in consequence of differences in turgidity, quite unlike that possessed by any other cells of the body of land plants.

Before any reasonable comparison of the results of Darwin, Lloyd, and ourselves may be made we should note the conditions under which the particular plants studied were grown, as well as the methods used in securing the different results. The conditions of the three sets of experiments present two extremes and a favorable mean. The ivy and laurel which Darwin used were growing in the moist climate of eastern England: the ocotillo and verbena of Lloyd are typical xerophytes of the Arizona desert. Ocotillo, or "coach-whip cactus," is lithe, slender, unbranched, and in appearance and structure like the cactus among which it grows. Verbena is a hardy perennial, persisting through the annual period of drought by means of its deep roots and withering stems. Intermediate between the extremes of England and Arizona are the climatic conditions at Stanford University, California (6), where the humidity is fairly high at all times, and where the temperature, while varying considerably, very rarely reaches an extreme of heat or cold. At times the vegetation is subjected to conditions as moist as those of England. This is true of certain rainy seasons particularly, and we find here a rainy season flora, typified by *Montia perfoliata* (Donn.) Howell, very different from that of the dry season, of which the Hemizonias may be taken as characteristic.

It is well known that plants develop more or less different structural characters in different environments. Where the humidity is highest, the cuticular covering of the epidermis is thinnest, other things being equal; and where, as in the deserts of Arizona, the humidity is low and the temperature high, the cuticula is thickest. It is important to bear this in mind, since it is obviously possible that, in certain circumstances, cuticular transpiration or evaporation may take place. While it is true that the cuticula protects the underlying tissues from evaporation, it does so only incompletely. Stahl's cobalt chloride test (7), which is purely qualitative, is not delicate enough to demonstrate cuticular evaporation, or cuticular transpiration: but the method of Buscalioni and Polacci (8) may be employed to show that cuticular transpiration may take place in addition to that through the stomata. Thus on applying a film of collodion to the epidermis of a leaf, through which the details of the leaf could be seen, it was found that clouding took place between the stomata as well as over them, though of course not necessarily at the same rate. Applying this fact to the plants with which Darwin experimented, it must be seen that a part of Darwin's error in recording transpiration was due to the cuticular evaporation of which the plants of his region are certainly capable. The cuticular evaporation of Lloyd's plants, on the other hand, would be very slight, while the Gramineae studied at Stanford University, with their moderately cutinized epidermis, exhibit a certain amount of cuticular transpiration. The regulation of transpiration seen to be effected by the stomata would lead one to conclude that they directly and mainly control the exchange of gases and the outgo of water. Only under exceptionally dry conditions would evaporation of water and the transfusion of gases through the outer walls of the epidermal cells be of any considerable importance. This we shall show shortly.

Furthermore, the density and the humidity of the air determine the intensity and the composition of the light reaching the earth's surface in different regions. Unfortunately it is still impossible to express these differences in definite terms, there being no single instrument or set of instruments which will give us all the data involved; but we know that on a hazy or cloudy day there may still be enough light to affect plants noticeably, though the sun's rays do not reach them directly. We are forced to conclude that the quantity of light reaching the earth's surface is less in Cambridge, England, because of the higher humidity of the air, than at Stanford University, and still less than in Tucson, Arizona. Some of the bearings of this inequality of light distribution have been already discussed by one of us elsewhere (6); it must be distinctly borne in mind in connection with the study of stomatal behavior. For example, the occurrence of high fogs at night and in the early forenoon in the San Francisco Bay region marks an important difference in the illumination as compared with that where Lloyd worked.

Darwin measured the amount of transpiration and the corresponding changes in the stomata by means of a "poremeter" fitted directly upon the surface of the leaf. The variations were estimated from the rate of flow of a current of air drawn through the stomata of an uninjured leaf under a given pressure. The leaf was supported upon a glass plate and the poremeter placed on a washer or perforated disc fastened to the leaf by means of gelatine. Others used paraffine, and also illuminated the leaf from below. Obviously, stomata enclosed within a poremeter are shut off from both light and air; CO_2 necessary for food manufacture is shut off during the progress of the experiment, and the leaf undergoes a sweating process in which water is given off but nothing is taken in, which would very rarely, if ever, take place in nature.

Lloyd, on the other hand, used two methods of experimentation, of which one was more natural and satisfactory than the other. His early method involved the measurement of stomata in strips of epidermis torn from leaves and fixed in absolute alcohol. Although there are many evident advantages in this method, even its convenience was never sufficient to convince everyone that there was no change whatever in the stomata by the most deft stripping of the epidermis from the surface of a leaf and the dropping of the strip into absolute alcohol. These objections are all avoided, though others may be raised, by making direct observation and measurement of the stomata in position on the living leaf, still attached to the plant which bears it. By attaching a Soyka flask, filled with water or an aqueous solution of suitable composition, to the under side of the stage of the microscope so that the light is cooled before it reaches the leaf to be examined, one may directly observe the condition and the changes in the position of the guard cells as the illumination changes.

We used this latter one of Lloyd's methods, but only on hot days did it seem necessary to us to apply the cooling cell. Measurements of the stomata on the leaves of plants of wheat, oats, rye, and barley, growing out of doors or in pots in the greenhouse, were made by bending over a leaf and gently applying and fastening it to the stage of a horizontal or other microscope for a minute, or a minute and a half, during which time the part of the leaf in the field was subjected to observation. The measurements were made by eye-piece micrometer, and made and recorded as rapidly as possible, so that no injury to the leaf and no change in position of the guard cells followed the slight darkening under the microscope. Most of the readings were made by daylight; but the few night readings showed the stomata to be closed in darkness. Measurements were made from two or three leaves on each plant for each period of time, and the average mean was taken in plotting each point; for in a group of plants there would always be some variation in the amount of light received by each plant and each leaf, both as to the times at which the most light would reach them and as to the quantities to which each would be exposed. In choosing the parts to be examined care was taken to select those leaves and those parts of leaves which were most isolated and most subject to variation at the particular times of examination. When the illumination is fairly equal for all the plants and all the leaves in a pot or box of plants of the same species, the condition of all the stomata is similar. This is not the case when the exposure is not similar. In cases in which the plants and their leaves were not similarly exposed all around, the turning of the plants so that they were similarly exposed was followed by the corresponding changes in the guard cells. No attempt was made to secure a series of readings for the early morning hours, or after sunset, because we were concerned primarily with the behavior of the stomata in relation to natural light; and we believe that intense artificial light disturbs and perhaps interferes with the natural action of the guard cells.

Temperature and humidity readings for each examination of the stomata were made. The latter readings were on a Mason hygrometer, which consists of a wet and dry bulb thermometer and is supplied with tables indicating the humidity at the different readings. Soil samples were taken from each box of plants, weighed, dried to constant weight, and the percentage of soil moisture was calculated from these figures. Darwin and Lloyd appear to have worked with mature plants and to have had them under fairly constant conditions. We have used the domestic grains, wheat (*Triticum vulgare* Vill.), oats (*Avena sativa* L.), rye (*Secale cereale* L.), barley (*Hordeum sativum* Jess.); and the wild oats of this region (*Avena fatua* L.). Plants of each species have been studied under three sets of soil conditions, namely (1) with the soil moist, (2) with soil saturated by watering, and (3) with the soil dry and often caked. They have all been examined under such different atmospheric conditions as hot, bright days, cloudy and very dark days, and days of light rain. Young, mature, and older plants have been studied, each being subjected to the same environmental factors whenever that was possible. The degree of illumination and the percentage of soil moisture have been recorded for each reading. Most of the experiments were conducted in a greenhouse with frosted roof but clear end. Sunlight is reduced, but only slightly, by these means which, in our climate, are indispensable. As a check upon this work in the greenhouse, we cultivated the same domestic grains in the Experimental Garden, and examined them there, under conditions as nearly as possible like those of the greenhouse. The wild oats were studied in the field and also in the greenhouse, into which plants of various ages had been removed from time to time in order to permit them to adjust themselves to the new conditions.

These four grains react similarly under like conditions; but each species displays individual differences which may be distinguished, and which partly explain the differences in needs and behavior which are familiar to the practical farmer. We shall discuss these differences after each species has been reported upon. Barley, wheat, and oats live under conditions of soil and of moisture essentially similar, while rye is best grown in drier and warmer localities. When planted in a fairly humid greenhouse it presents a somewhat different appearance from the normal and from that of the other grains. These things are indicated by the details which follow.

Barley

Figure 1 indicates the behavior of stomata of barley seedlings eighteen days old and grown in the greenhouse. The soil was very wet in the early morning, and no more water was added during the readings. The temperature was fairly high, but increased, with the increase in light, to a maximum of 34.5° C. at 1 P.M. The humidity reached its lowest point at 3:20 P.M., when it was 33%. The stomata opened gradually in the morning until 11:50 A.M., when they reached their maximum width for the day.

At the same time the sunlight attained its maximum brightness. In this figure it is indicated by curves that the light intensity and the opening and closing of the stomata vary similarly, whereas the humidity curve goes in the reverse direction. The curves are made of broken lines, as follows: the *continuous* lines connecting the small circles form the curve indicating the opening and closing of the stomata; the *broken* lines of pieces of *equal* length between the small circles form the temperature curve; the *broken* lines of *two unequal* lengths connecting the small circles form the humidity curves. The figures at the bottom of the diagram indicate the hours from 8 o'clock in the morning to 6 o'clock in the evening. The figures at the left indicate the temperature in degrees Fahrenheit because our Mason hygrometer was supplied with Fahrenheit thermometers. In words we have stated the temperature in degrees Centigrade. In the graphs the difference is *nil.*

Figure 2 indicates the behavior of the stomata of barley seedlings twenty-four days old, with 11.3% of moisture in the soil at 9:25 A.M. and 10% at 4:30 P.M. The loss of water from the soil during this time from the surface of the soil and from the plants, through their stomata and otherwise, was 1.3%. The temperature of the air decreased slowly from 84° F., 28.9° C., at 2:40 P.M., and the humidity rose from 62% at 9 A.M. to 72% at 2:40 P.M., from which time the two curves converged somewhat. Thus the atmospheric conditions of this day were moderate, and represent a mean average condition as compared with those of the previous day. At 9 A.M. there was a heavy fog but nevertheless enough light to cause the stomata to open slightly. At 11 A.M. there was a short time of fairly bright light, but a time of comparative darkness followed, and at 2:40 P.M. the stomata were all but closed. At 4:15 P.M., when it was almost too dark for further readings, the stomata were all closed.

Figure 3 presents a contrast to the case just described. This is the record of a similar box of barley seedlings, thirty-seven days old. The soil was moist at 9 A.M. but was watered. At 3:40 P.M. it showed 21.7% of moisture. In the last case a heavy fog prevailed during the early hours of the morning, but there was a fair amount of light. In the present instance the day was cloudy and, for this country, very dark until 2 P.M., when there was a brief interval of light, but by 3 P.M. the dusk of evening had begun. The stomata remained closed throughout the day, as the straight line shows. The soil was moist and the plant cells were turgid, but with light insufficient to stimulate the guard cells the stomata remained closed. The slight amount of light at about 2 P.M. was not enough to affect them. The temperature reached 28.9° C. at 11.55 A.M., and the humidity fell to 47% at 2 P.M., the curves of temperature and humidity showing a rather wide divergence in the early afternoon hours and a close convergence, as indicated before, at 4:55 P.M.

WHEAT

Similar measurements were made of wheat stomata, and the data follow. Thus figure 4 shows stomatal and other measurements taken in a box of wheat seedlings twenty-three days old. The conditions were nearly the same as those described in connection with figure 1, and there is much similarity in the diagrams. The light was bright from the beginning of the day, and was intense between 12:30 and 2:30 P.M. The maximum temperature was 38° C. at 1:15 P.M., the maximum humidity 57%. The humidity fell to 46% at 2:15 P.M., but rose slightly from then on with the decrease in warmth. The soil moisture was 20.4% at 8:25 A.M. More water was added and the soil kept very wet all day. With this abundance of moisture and light the stomata opened, but they did not respond as rapidly as the barley, as shown by the slower start. The period of greatest expansion of the stomata was shorter than in barley, and the period of closing was also shorter. The temperature and the humidity both remained high as late as 4:15 P.M., when the stomata almost closed.

The degree of turgidity of the cells of a plant depends, other things being equal, upon the percentage of moisture in the soil in which they grow. Without considerable turgidity the guard cells shrink or collapse. In this condition light does not so stimulate them that they open. This fact is indicated by wheat seedlings, the minimum water requirements of which, under the conditions of our experiments, were found to lie between 16.7% and 17.8% of soil moisture.

Another set of wheat seedlings thirty-seven days old, grown in 17.8% of soil moisture, illustrate the intimate relation between light and the opening of the stomata. The morning of observation was dark and cloudy until 10:30 A.M.; then there followed an interval of weak light to about 1:30 P.M., a cloudy period at 2 P.M., a clearing of the clouds at 3:00 P.M., and the dusk of evening coming on at about 4:45 P.M. The stomata opened slightly during each light period and closed with each recurring darkness, and thus opened twice during the single day. The temperature and the humidity were high throughout the period of the readings.

In figure 5 no such sensitive response to light is recorded. Here are shown the records of wheat seedlings fifteen days old, with soil moisture at 16.7%. The plants were erect and showed no outward signs of wilting, but the guard cells appeared to be somewhat collapsed. The temperature rose to 34° C. at 2:05 P.M., and the humidity rose slightly in the forenoon and fell in the afternoon to 42% at 3:15 P.M. The sun shone brightly at 8:30 A.M. and the sky was cloudless throughout the day. External stimuli were such in this instance as to indicate a wide opening of the stomata; but, because of the shortage of soil water, they remained tightly closed throughout the day, protecting the leaf from evaporation and thus conserving such moisture as it contained. In the preceding case, as in this one, there is a striking similarity between the humidity curve and the stomatal curve.

As the humidity began to fall the stomata began to open: but as the curves show, the stomata did not begin to fluctuate with the changes in humidity, but rather were guided in their movements by the amount of light. Fall of humidity, therefore, is probably not a factor influencing the behavior of the guard cells.

Figure 6 is representative of many sets of wheat seedlings examined on moderately bright days with varying light intensity. In this case the light was brighter than in the two preceding. The plants were ten days old, and the soil moisture in the box varied from 20.9% at 9:25 A.M. to 22.2% at 4:30 P.M. The temperature and the humidity both remained within the limits of 76° and 64%, converging very closely at 4:15 P.M. Conditions were such as to keep the stomata open all day, although a dark period about 11:30 A.M. caused a partial closing, as indicated by the drop in the line in the figure. By 2 o'clock the light was strong enough to cause the maximum opening for the day, as shown by the stomatal curve.

Oats

The reactions of the stomata of oat seedlings very closely resembled those of barley and wheat. That there are special differences, however, is revealed by the accompanying graphs. On examining an extensive series of stomatal reactions, figure 7 was chosen as indicating the optimum condition for oats. The soil was comparatively dry at 8:30 A.M., there being only 9.9% of soil moisture, and no water was added during the day. The temperature rose to 35.5° C. at 1:30 P.M., and the minimum humidity, 44–46%, prevailed between 12:45 and 2:55 P.M. The seedlings were fifteen days old and well developed. The sun was unclouded from 8 A.M. on the day of these readings, and the light reached its greatest brightness between 12 o'clock noon and 2 P.M. The first readings showed the stomata open. They continued to open still wider until the maximum was reached at 12:45 P.M. The maximum width was maintained for a few minutes only, after which they began to close. The closing was very gradual, however, as they were still slightly open at the last reading, which was at 4:30 P.M.

When one compares this curve with that in figure 1 for barley and that in figure 4 for wheat, all representing optimum conditions for their respectives species, it is obvious that the stomata of oats, when they have reached their maximum expansion, take longer to close, as well as to open, than do those of the other two plants. Their guard cells react less promptly to light stimuli than the guard cells of the stomata of barley and wheat. Of all the grains examined, wheat stomata are the ones most sensitive to light, if one may judge by the speed of reaction of the guard cells.

To determine the amount of soil moisture necessary for oats, two boxes of plants, each sixty-three days old and very similar in character, are used. At 9 A.M. both contained 8% soil moisture, and each was abundantly watered. The soil was slightly caked and the plants showed some wilting. Figure 8 represents box A which, after watering, was placed out of doors in very bright sunlight. The humidity fell rapidly and remained low for the day, varying only 4% from 27% at 11 A.M. to 4:10 P.M. The temperature reached 32° C. at 11 A.M. and gradually fell to 61 at 4:10 P.M. By 1 P.M. the wilted plants had become erect and the cells had assumed a more nearly normal turgescence. By 4:10 P.M. twilight had come on, and the stomata remained closed thereafter.

Figure 9 represents the similar plants in box B, treated in like manner except that they were left in the greenhouse. Although the plants in this box did not exhibit such extreme wilting as those in box A, nevertheless the guard cells proved, on examination, to be completely collapsed. Light was abundant throughout the day. By 1:40 P.M. the guard cells had recovered the necessary degree of turgidity and began to open steadily until 4:10 P.M. After this hour the light was too dim for readings.

These two cases, therefore, show that both conditions must be present together in order that the guard cells may open. The cells must have reached their full degree of turgidity before light will affect their action; and the more turgid the cells are at the time of exposure, the more responsive they will be to the different degrees of light which reach them. Comparing the soil moistures of boxes A and B with that of figure 7, it is found that the minimum moisture requirements of these oats lay between 9.9%, a favorable condition, and 8%, a wilting condition.

RYE

Rye seedlings of different ages exhibited differences in the stomatal reactions of the young and the old plants. In the three grain species already described there is no difference in the behavior of the stomata of the plants of different ages; all reacted in the same way under the same stimuli. As already mentioned, rye thrives best, for economic purposes, where the soi and air are relatively drier than where the other grains thrive best. Thus our rye grew poorly in the humid air of the greenhouse, but finally matured into somewhat stunted plants.

Figure 10 gives the stomatal curve for rye seedlings fourteen days old, on a very bright day, with soil moisture at 10% at 9:45 A.M., and with no more water added during the day. The temperature reached 36° C. at 1:40 P.M., and the humidity remained high throughout the day. The stomata opened at 8:50 A.M. and remained but slightly open until 1:30 P.M., when they closed for the day. Sunlight continued until after 4 P.M. This and other similar cases showed that the stomata of young rye seedlings open to a very slight degree only.

Rye plants seventy-two days old and beginning to bloom are indicated by figure 11. At this time the soil moisture was 20.9% and the temperature reached 32° C. at 1:30 P.M. The humidity remained stationary at 31%

from 11:50 A.M. to 2:30 P.M. The light was bright from 8:30 A.M. till 1:30 P.M. From that hour it waned until twilight, which came on shortly after 4 P.M. The stomata of these older plants more noticeably responded to the light than did those of the younger plants described immediately above. They opened at 9:30 A.M. and remained open until 1:30 P.M.; but though their width was uniform throughout this time, they were not as widely open as the stomata of the other grain plants. Nevertheless, in the case of rye also, the width of the stomatal opening corresponded to the intensity of the sunlight. The fact that the stomata never opened to their full width may be an important and significant adaptation, or reaction, to their environment. If, for example, the stomata opened wide where the air is dry, transpiration might be so excessive as to dry out the plant and destroy it. One of us has shown that when the guard cells are killed or paralyzed by sulphur dioxide fumes, the rate of transpiration goes up, other things being equal, and the plant, the organ, or the part may dry out and die, because the plant has lost control, locally or generally, of its water.

The minimum water requirements of rye were not especially studied; but our observations indicate that they are below 10%. Rye plants appeared to develop quite favorably on soil containing 10% or less of water, whereas if the amount reached 20% or more the plants did poorly.

To provide a check upon the previous work we conducted the following experiments upon these four species of grain grown in pots. One pot of each species was placed in bright sunlight and one pot of each was put into the dark. The temperature and the humidity were kept the same for both sets by means of an electric fan. In all cases the stomata were slightly open when the experiment began. The results were so similar in all four species that only those obtained from the wheat plants will be reported here. Wheat is selected for this because we were able to reverse the positions of the pots, in the case of the wheat, on the following day, thus giving us a check on the check furnished by the first set of experiments. Figure 12 gives the two very dissimilar curves constructed from the records of the behavior of the stomata of wheat in light and in darkness. Temperature and humidity were both high, and there was an abundance of moisture in the soil. At 8:30 A.M. both sets of plants were examined. The stomata were open. Each set was watered and one, A, placed in the light, the other, B, in darkness. The rise and fall of the line A in figure 12 corresponds with and indicates the increase and decrease of sunlight during the day. B shows that the stomata closed at 10 A.M. and so remained as long as they were in darkness. Figure 13 records the behavior of the same two pots of plants, B put into the light, and A into the dark. Conditions of light and of soil moisture were very similar to those of the day preceding, but the plants were subjected to the opposite effects of light and darkness. Hence the stomata of the plant placed in the dark closed promptly and remained closed, whereas those of the plant which was brightly lighted continued to open. Hence, the behavior on one day, in one set of conditions, was the opposite of the behavior on the following day, under conditions exactly the reverse so far as light was concerned but similar in every other respect.

To answer the question what effect, if any, the conditions in the greenhouse exerted upon the behavior of the stomata of these four species of grain plants, we grew another set in the open, in the Experimental Garden which now forms part of the equipment of this laboratory. When fifty-five days old, on December 29, they were examined. The results are indicated in figure 14. The day followed a cold frosty night, but was very clear from early dawn. The sun appeared at 8:30 A.M.[1] The humidity fell to 57% at 1:15 P.M., but it remained much higher during the rest of the day, both earlier and later. The temperature was about 15.5° C., ranging somewhat above and below this figure. It is obvious too that, at this season of the year, even in a latitude no further north, the position of the sun results in a light intensity considerably less than at other seasons. Nevertheless, it will be seen, on comparing the stomatal curves in this figure, 14, with those of the same species previously reported and experimented upon under glass, that there was little or no difference in the general behavior of the stomata. At 2 P.M. the light began to fade and all the stomata began to close. Of the four, the stomata of wheat closed soonest, the other three closing at about the same time and rate, as the line Y-Y′ shows. These results indicate plainly that, so far as stomatal action in response to light is concerned, there is little or no difference in behavior due to the effect of the greenhouse.

WILD OATS

While the experiments above described were in progress it was suggested that we ascertain the behavior of the native wild oats (*Avena fatua* L.), which were growing in the Experimental Garden. Several plants were potted and taken into the greenhouse. Figure 15 indicates the stomatal movements on a young and on an old plant on the day following transplanting. The soil was kept very moist, and the light was moderately bright. The stomata on the young plants remained open during the brightest part of the day, from 9:15 A.M. till 2:05 P.M., but on the old plant they failed to open.

Mature plants of wild oats tend to open their stomata somewhat during the early morning hours, and then to close them for the remainder, and the hottest part of the day. Figure 16 shows this action on a very clear, bright day, with the temperature at 23° C. and the humidity at 43% during the middle of the day with the soil moisture at 12%. Similar plants examined on days with high clouds, or with light rain, the soil moisture being about the same as before, namely 12%, showed that the stomata remained open throughout the day. In this respect there was more variety of action among the older plants than among the younger ones. Thus figure 17 records the behavior of two such plants, *a* young, 4–8 inches high, and *b* a plant in bloom, under the same conditions and on the same day. The temperature reached 34° C. at 1:40 P.M., and the humidity remained almost constant, namely at about 60%. There was no direct sunlight during the day, but at times there were very bright intervals. Wild oats, therefore, are able to absorb CO_2 and to manufacture food on days when the other plants here reported upon would keep their stomata closed, because the stimulus required for their opening would be too weak to produce the needed effect. On the other hand, figure 16 indicates the behavior of a mature wild oat plant growing out of doors in the Experimental Garden. After a day of rain the moisture in the soil amounted to 12%, the sky was cloudless, and the sunshine correspondingly bright. Nevertheless, the stomata closed at the time of maximum illumination. This time was also that of maximum temperature and minimum humidity. Apparently we have, in this behavior of the wild oat out of doors, a decided contrast with the behavior of the cultivated grains. Its behavior in the greenhouse is similar, however, to the others. Out of doors the conditions of its existence are somewhat different from those ordinarily prevailing for the cultivated varieties, as the following description will show. The wild oat of California grows and fruits throughout most of the year, naturally reaching its best development during the wet weather of early winter and of spring. The leaves are slender, tough, thick, and hairy, well adapted to withstand severe drying of the soil; for this species grows commonly along dry roadsides and in open fields and pastures, where it is subjected to pronounced drying, perhaps more than once during its life. The specimens which we studied were well developed and in bloom during the early part of December. In midsummer and later the plant would not thrive, and only where there was some moisture would it hold out at all. On the other hand, the cultivated grains can be grown at any time of the year, providing there is sufficient warmth, moisture, and light. They are naturally spring species, however, and grow best with the warm rains, and fruit in the early summer. In these differences in habitats, and in the corresponding differences in habits, we see reasons for the behavior of the two sets of plants.

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[1] It should be stated that, owing to the two mountain ranges bounding the Santa Clara Valley to the eastward and the westward, sunrise and sunset are respectively later and earlier than they would be on a wide plain in the same latitude. Sunset and darkness come especially earlier in our laboratories because Stanford University is built at the foot of the hills which rise to the mountains to the west.

That the behavior of *Avena fatua*, in the respects in which it differs from that of the four cultivated grain plants which we studied, is the product of circumstances is indicated by figure 18, in which the movements of the stomata of wild and cultivated oat plants are recorded. In this experiment in which wild oats, transplanted from a field and kept in the greenhouse for thirty-five days, were compared with cultivated oats, we find the following circumstances. The temperature was moderate throughout the period of observation, the maximum being 27° C. at 1:10 P.M.; the humidity was lowest at the same hour, namely 49%. In the figure line (*a*) represents the behavior of stomata of cultivated oats, mature plants seventy days old, in soil containing 24.5% of moisture. Line (*b*) indicates the behavior of the stomata of a large plant of *Avena fatua*, then in bloom, which had been in the greenhouse for thirty-five days, in soil containing 15–20% of moisture at the time of observation. No water was added to either box during the day. The sky was clear and the light bright from 8:00 A.M., reaching its maximum brightness between 11:00 A.M. and 1:45 P.M. At the latter hour the sky clouded, but by three o'clock it was lighter again. The sun set behind our mountains at four o'clock, and the plants were in shadow from that time on. The two curves show that the stomata remained open as long as there was bright light; that, on the dimming of the light soon after one o'clock, the stomata of the cultivated species closed and did not open again during the rest of the day, although the light later on became somewhat brighter again. On the other hand, the stomata of the wild oat closed very gradually, and they were completely closed only at sun-down. The stomata of the wild oat reacted to the dimming light, but only much more slowly than those of the cultivated species.

Wild oats growing in hard, dry soil were examined on various days. The plants were usually erect and exhibited no outward microscopic signs of wilting, but the stomata were shrunken and showed no movement. These wild plants survive in such dry surface soil, and on hot days on which the cultivated species wilt or burn, even though abundantly supplied with water in the soil. In the behavior of the stomata of these two species of oats we see one reason for the differences in their occurrence and in their requirements. Thus, the wild species can and does maintain itself in changing conditions in which the temperatures, humidity, soil moisture, and illumination may cover a very wide range; whereas the cultivated species requires a fairly high proportion of soil moisture, and it can withstand only moderate dryness of the air and moderate heat.

The Significance of Stomatal Movements

The foregoing experiments show that, in the species studied, there are definite times during which the stomata are open and other times during which they are closed. While the stomata are open carbon dioxide enters the leaves of these plants more rapidly than while they are closed, other things being equal. Furthermore, carbon dioxide will enter a green leaf not only in accordance with the openness of its stomata, but also in accordance with the rate at which it is being used in the leaf. This use constitutes the photosynthetic process resulting in the production of sugar, starch, etc., a process which goes on at rates proportioned, among other factors, to the intensity of the light. We see, therefore, that, so far as these five species of annual plants are concerned, a factor which regulates the rate of food

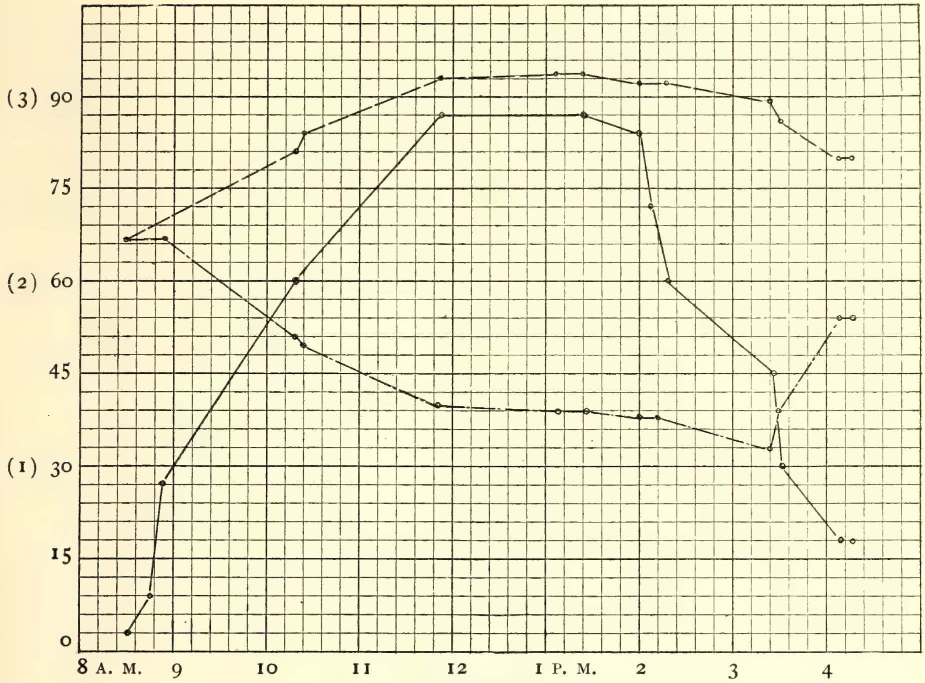

FIG. 1 (For explanation, see p. 155).

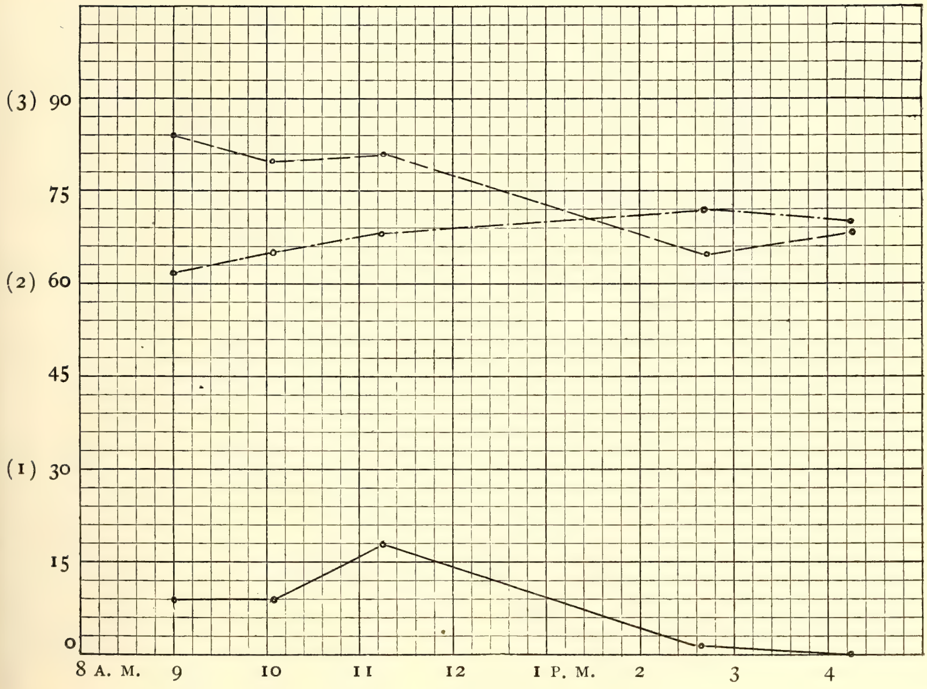

FIG. 2.

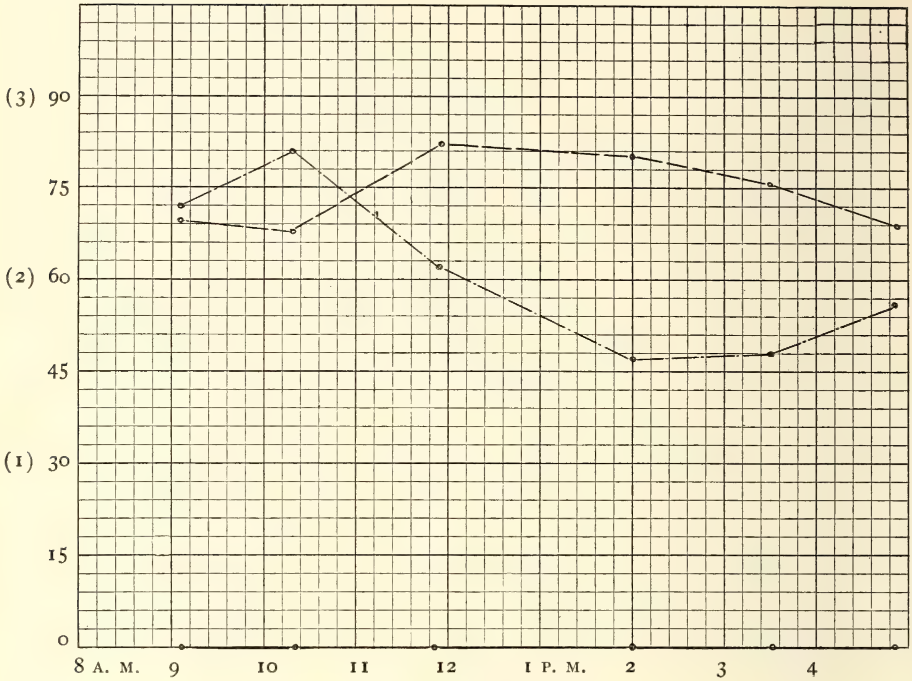
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 3.

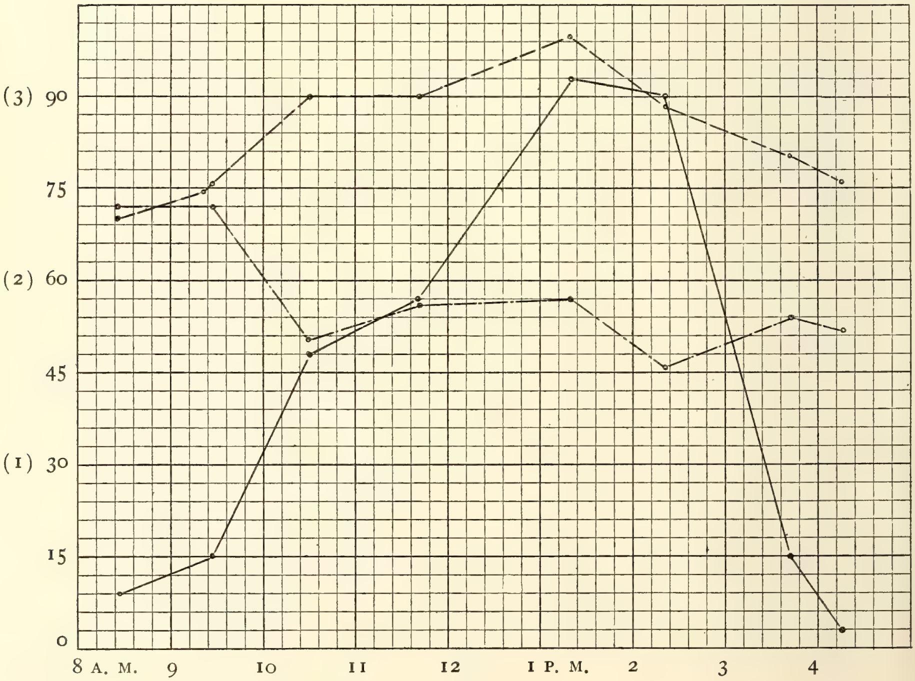
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 4.

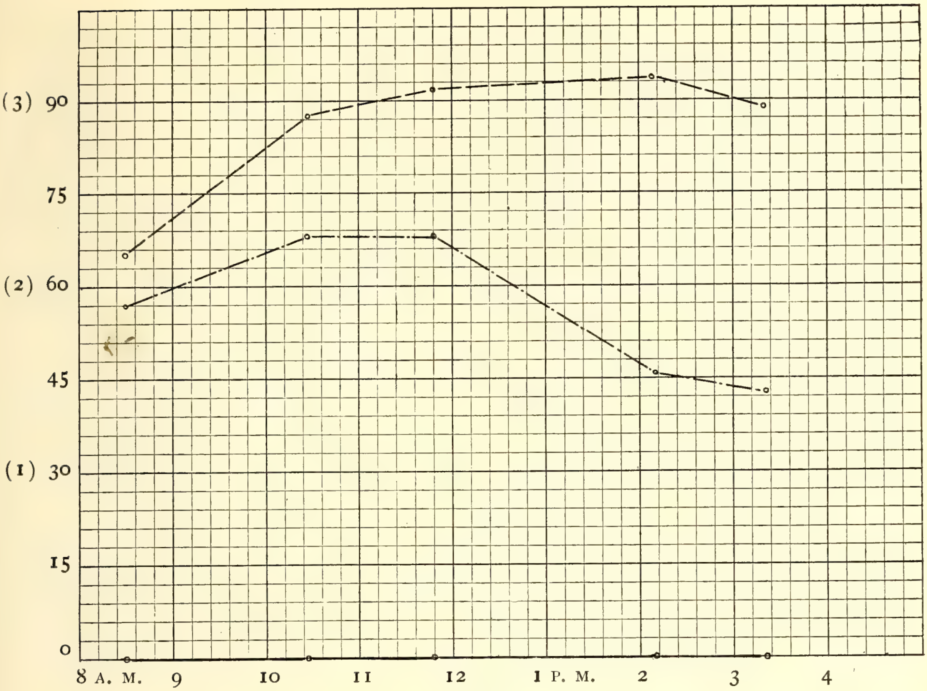
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 5.

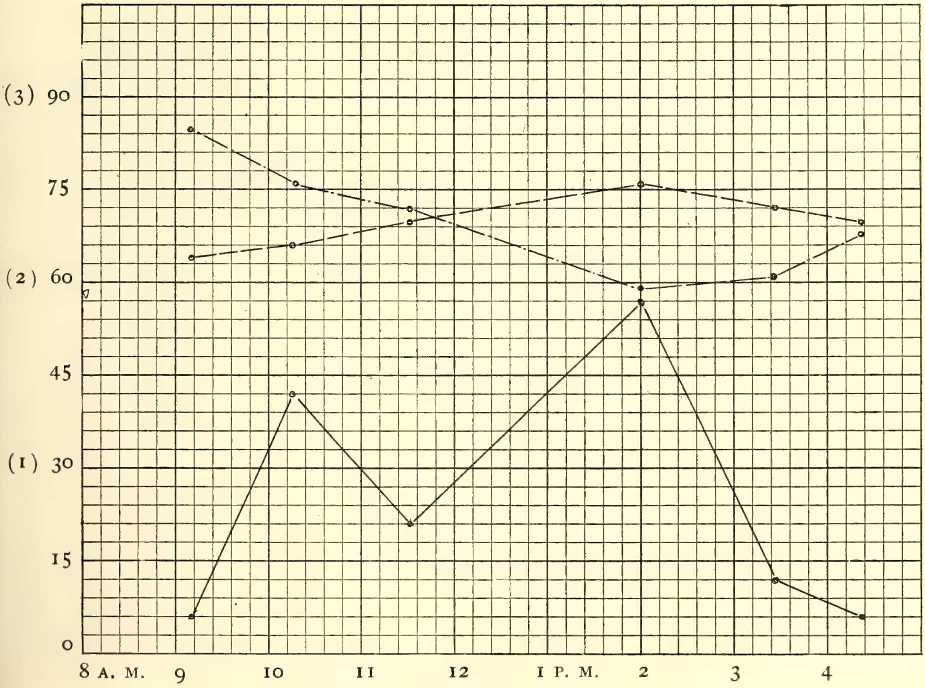
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 6.

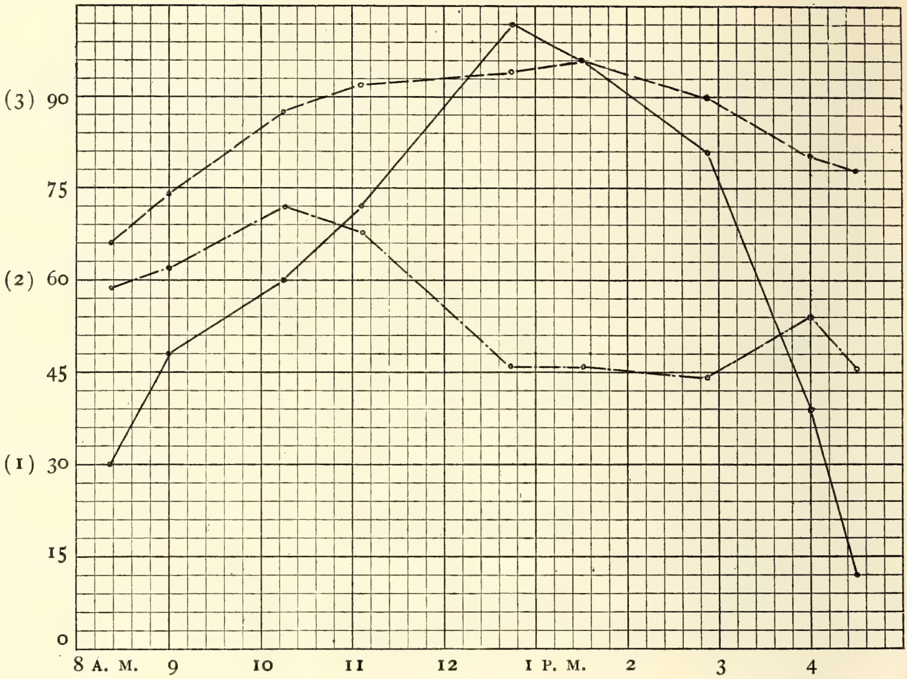
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 7.

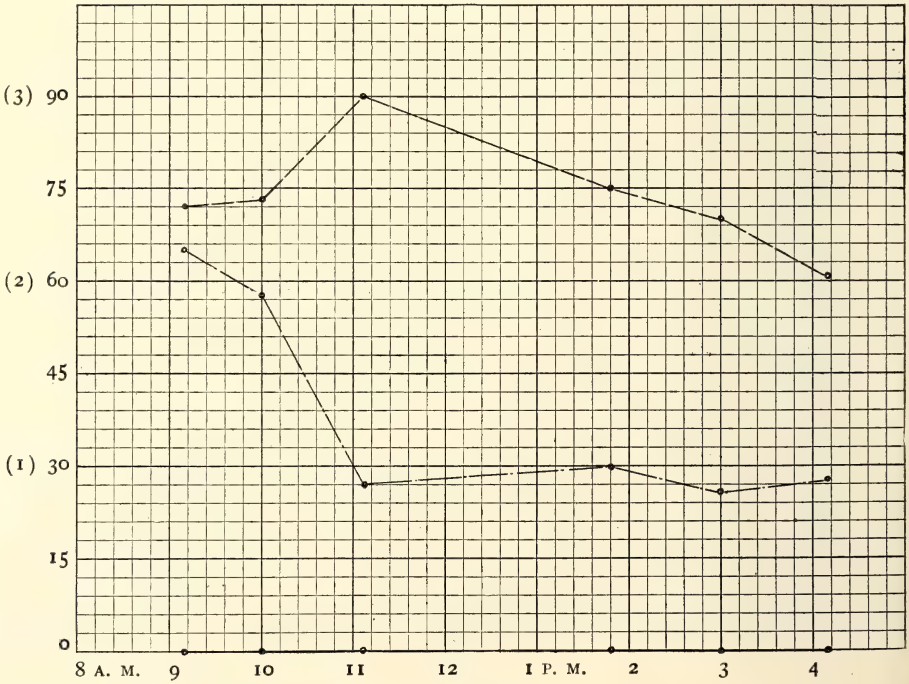
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 8.

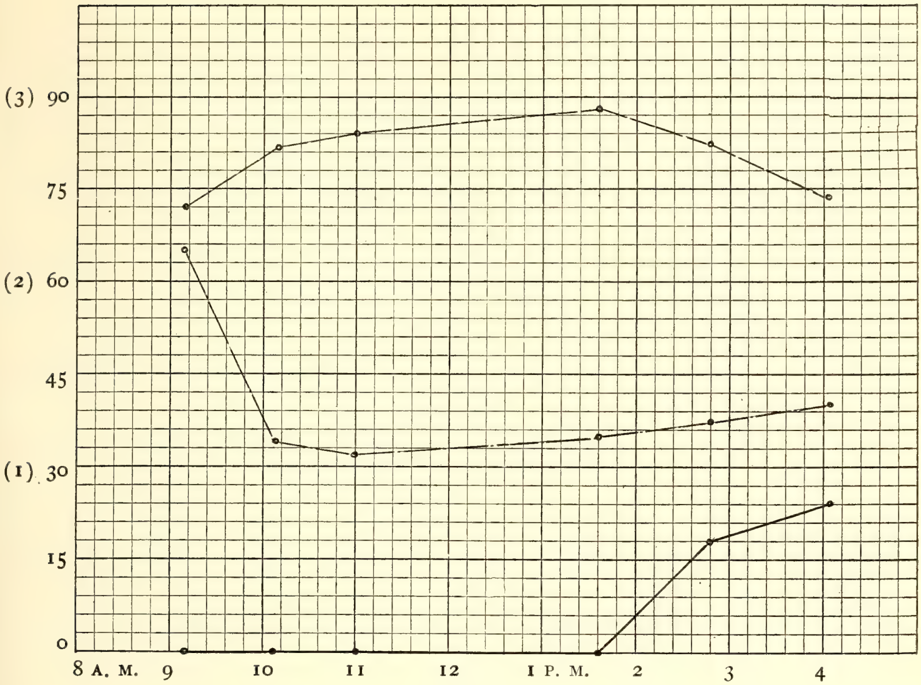
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 9.

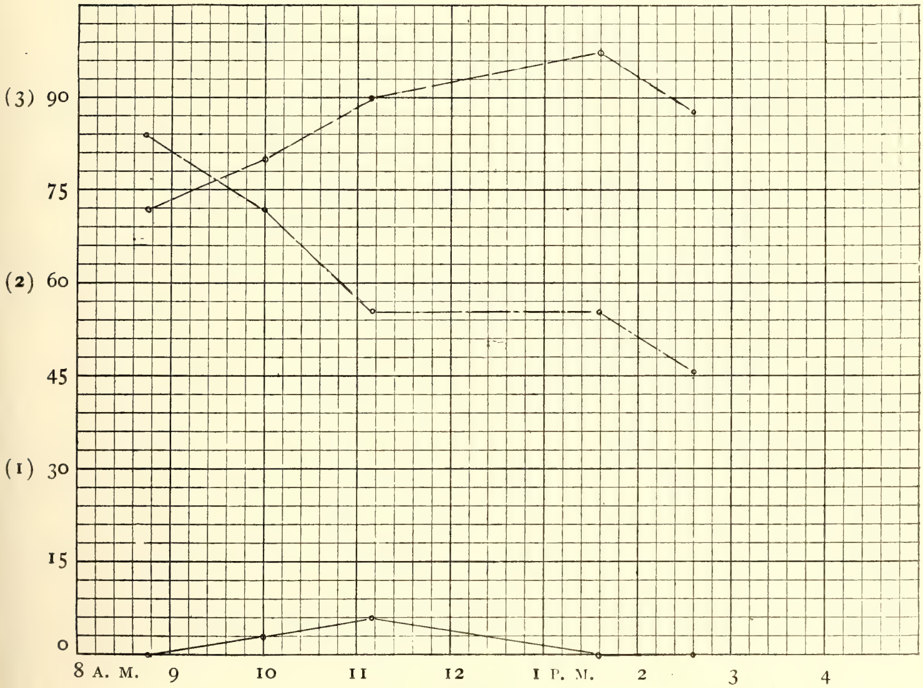
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 10.

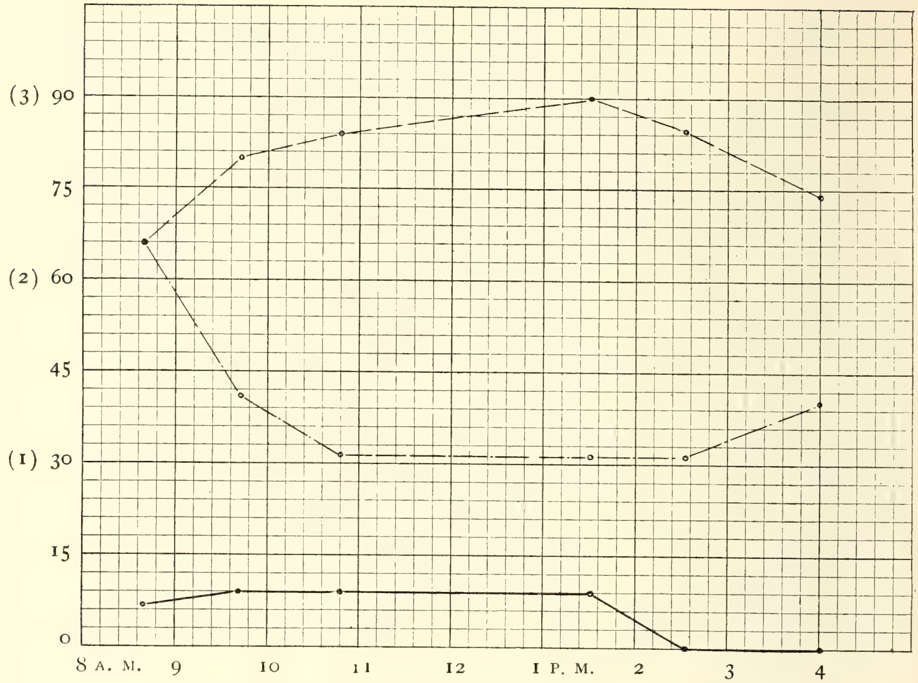
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 11.

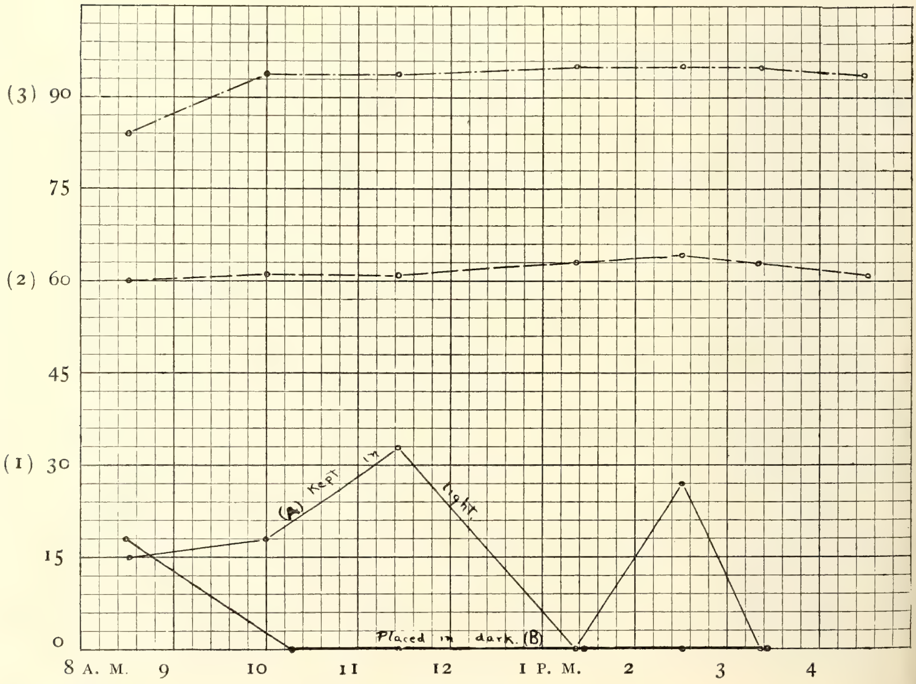
(3) 90
75
(2) 60
45
(1) 30
15
0
(A) Kept in light
Placed in dark. (B)
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 12.

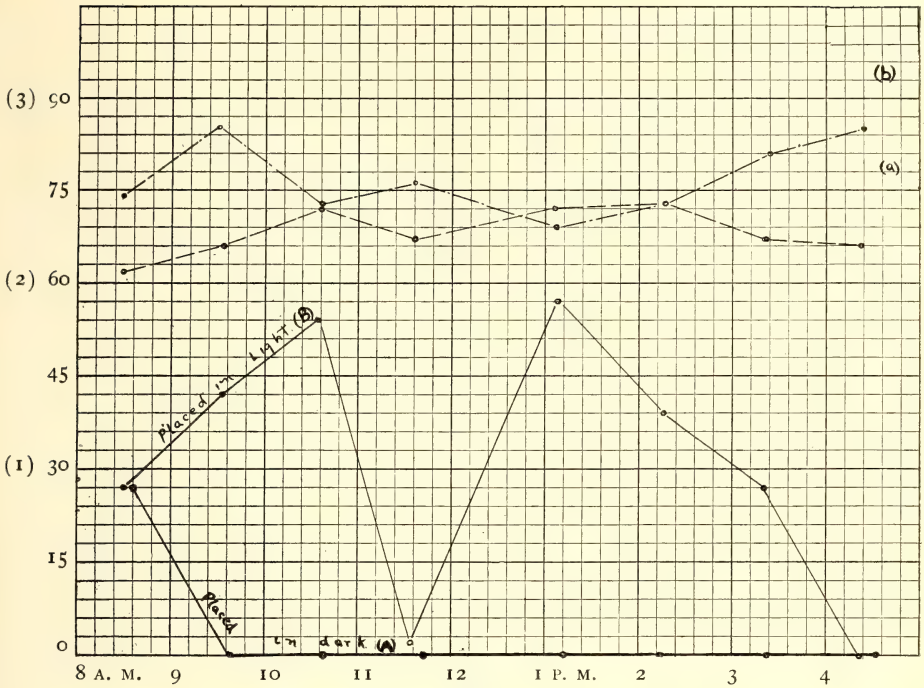
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4
(b)
(a)
placed in light (B)
Placed in dark (A)

FIG. 13.

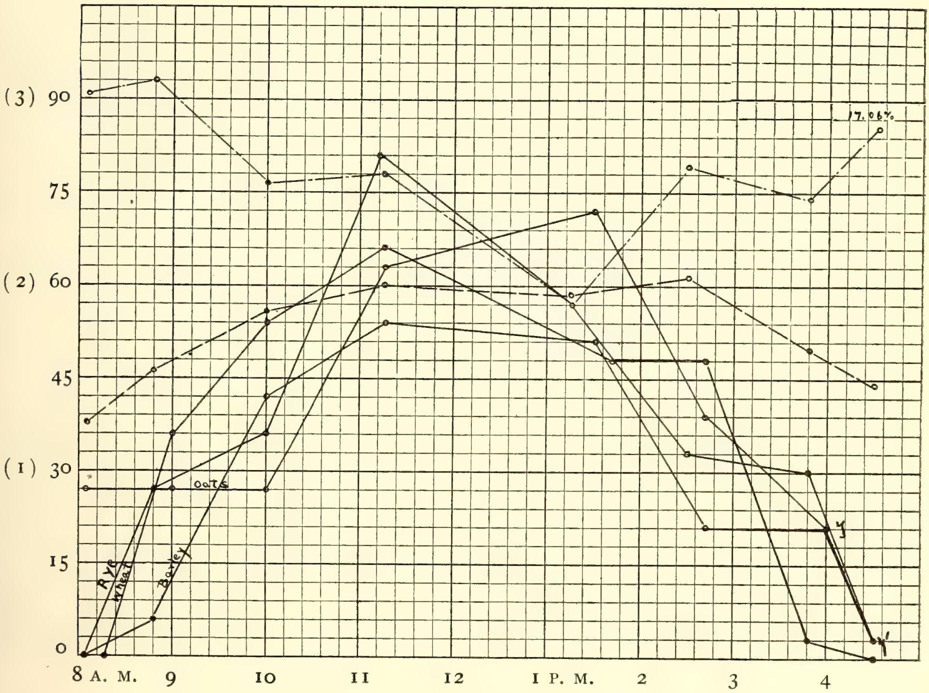
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4
17.06%
oats
Rye
wheat
Barley
y
y'

FIG. 14.

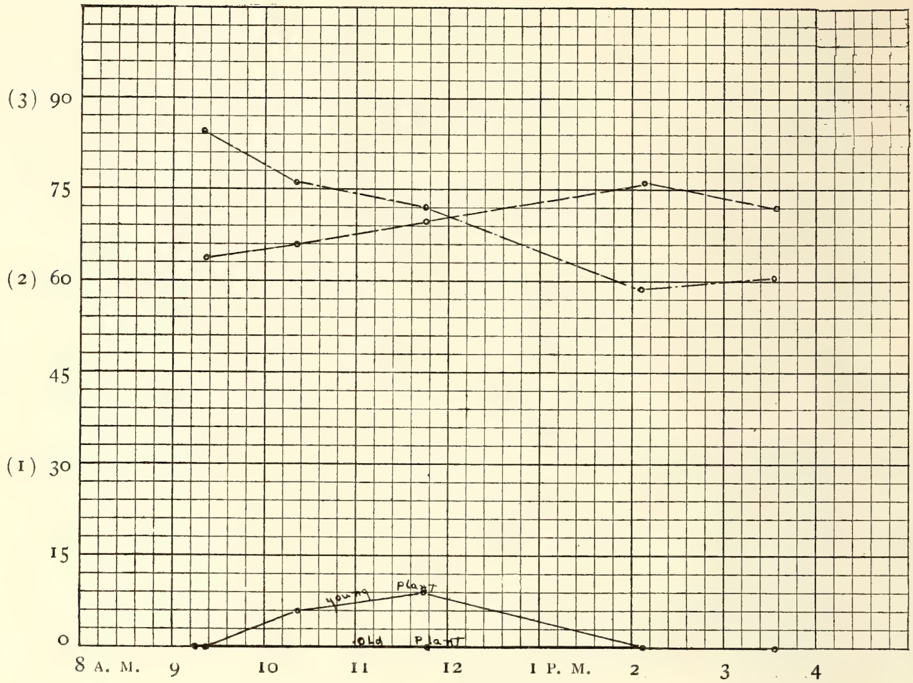
(3) 90
75
(2) 60
45
(1) 30
15
0
young plant
old plant
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 15.

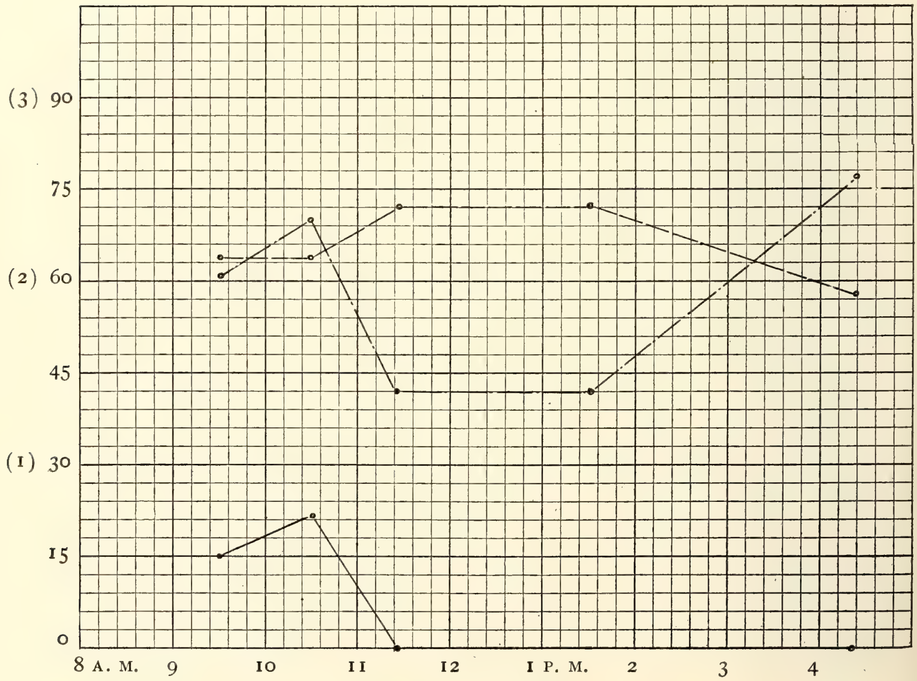
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4

FIG. 16.

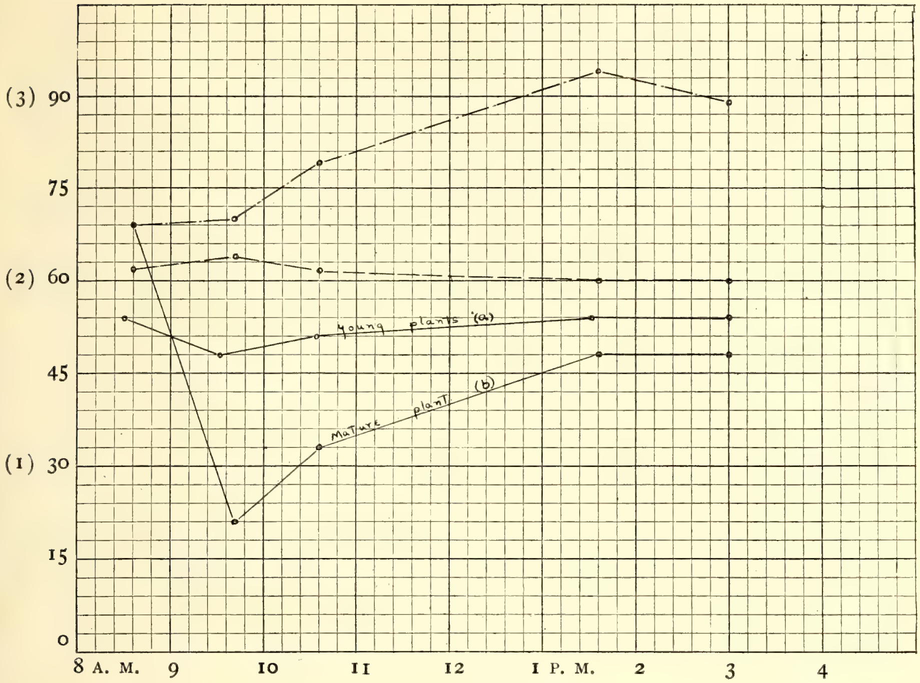
(3) 90
75
(2) 60
45
(1) 30
15
0
8 A. M.
9
10
11
12
1 P. M.
2
3
4
young plants (a)
Mature plant (b)

FIG. 17.

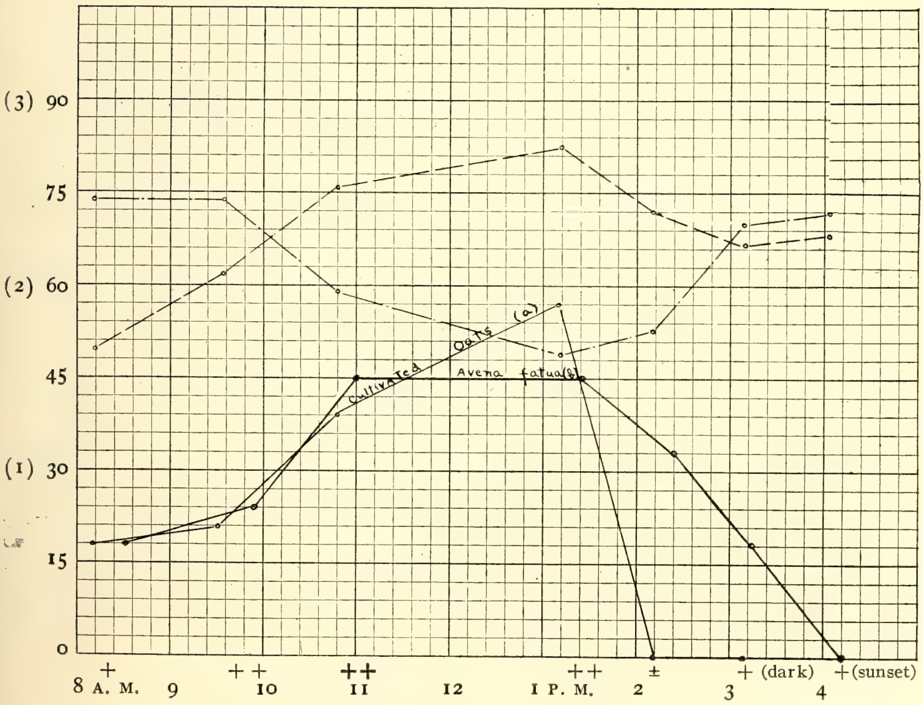
(3) 90
75
(2) 60
45
(1) 30
15
0
+
++
++
++
±
+ (dark)
+(sunset)
8 A. M.
9
10
11
12
1 P. M.
2
3
4
cultivated oats (a)
Avena fatua (b)

FIG. 18.

manufacture also regulates the opening and closing of those slits in the epidermis through which alone carbon dioxide can enter at a rate sufficiently rapid to supply this necessary raw material of food manufacture. This factor regulating both food manufacture and stomatal opening is light.

Conclusions

The study of the stomatal reactions of the cultivated and wild species of grains has led to the following conclusions:

1. The stomata of barley, wheat, oats, and rye plants open with light and close with darkness.
2. Increase or decrease in the amount of light, when it has reached a minimum intensity, will have a corresponding effect upon the width of the stomatal openings.
3. The opening and closing being accomplished by the changes in shape of the guard cells of the stomata, a minimum amount of moisture in the soil is required by each species in order to produce and maintain the turgidity of the guard cells without which changes in their shape are impossible.
4. The moisture, soil, and light requirements of the different species are essentially alike, though not identical.

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DESCRIPTION OF GRAPHS

In all the graphs the curves are made of broken lines. The different sorts are to indicate the following records:

Continuous lines connecting the small circles indicate the opening and closing of the stomata.

Broken lines of *equal* length form the temperature curves.

Broken lines of *two unequal* lengths form the humidity curves.

The figures at the bottom of the diagrams indicate the hours from 8 A.M. to 6 P.M. The figures at the left are those of the Fahrenheit scale, because our Mason hygrometers have only the Fahrenheit thermometers. The other figures to the left of the graphs (in parentheses) are the purely arbitrary numerals of the eye-piece micrometer used in measuring the widths of the stomata.

FIG. 1. Barley, September 16, 1916, age 18 days, bright sunlight, soil very moist.

FIG. 2. Barley, October 14, 1916, age 24 days, dark foggy morning, light toward noon, dull afternoon, 11.3% soil moisture at 9:25 A.M., 10% at 4:30 P.M.

FIG. 3. Barley, October 7, 1916, age 37 days, dark, cloudy day, light about 2 o'clock only, soil very moist at 9:05 A.M., then watered, 21.7% soil moisture at 3:40 P.M.

FIG. 4. Wheat, September 23, 1916, age 23 days, very bright day, 20.4% soil moisture before watering at 8:25 A.M.

FIG. 5. Wheat, September 24, 1916, age 15 days, bright day, 16.7% at 8:30 A.M., watered at 12:25 noon.

FIG. 6. Wheat, October 14, 1916, age 10 days, dark early, light later, 20.9% soil moisture at 9:25 A.M., 22.2% at 4:30 P.M.

FIG. 7. Oats, September 24, 1916, age 15 days, brilliant day, 9.9% soil moisture at 8:30 A.M., no water added.

FIG. 8. Oats, November 11, 1916, age 63 days, bright clear day, 8% soil moisture at 9:15 A.M., watered and put out of doors in very bright sunshine.

FIG. 9. Oats, November 11, 1916, age 63 days, bright clear day, 8% soil moisture at 9:15 A.M., watered and pot left in greenhouse in bright light.

FIG. 10. Rye, September 23, 1916, age 14 days, bright clear day, 10% soil moisture at 9:45 A.M. and not watered later.

FIG. 11. Rye, November 11, 1916, age 72 days, very bright clear day, 20.9% soil moisture, not watered afterwards, plant beginning to bloom.

FIG. 12. Wheat, October 21, 1916, age 17 days, foggy early, later bright; pot A, kept in light, 17.5% soil moisture at 8:45 A.M., watered, 23.7% at 4:00 P.M., difference 6.2%; pot B, kept in dark, 30.8% soil moisture at 8:40 A.M., watered at 8:45 A.M., 39.1% at 4 P.M., difference 8.3%.

FIG. 13. Wheat, October 22, 1916, age 18 days, light similar to that of preceding day, plants the same as preceding (figure 12), treatment exactly reversed, thus: pot B put into sunlight, 23.9% soil moisture at 8:40 A.M., watered at 8:40 A.M., very moist all day afterwards; pot A, kept in dark after 8:40 A.M., 21.7% soil moisture at 8:40 and then watered.

FIG. 14. Wheat, oats, rye, and barley, as indicated, December 29, 1916, growing out of doors in Experimental Garden since November 4; clear night with heavy frost at 8 A.M., light from daylight, sun up at 8:00, light brightest between 11 and 12, following by gradual dimming, 17.06% soil moisture.

FIG. 15. Wild oats, October 14, 1916, bright day, soil very moist; two plants, one young, first day after transplanting; one old enough for blooming.

FIG. 16. Wild oats, November 8, 1916, clear, very bright day with no clouds, 12% soil moisture, large plant in full bloom growing in Experimental Garden.

FIG. 17. Wild oats, November 4, 1916, cloudy with slight rain at intervals, light much brighter than could be expected with clouds, etc.; two sets of plants, one young, 4–8 inches tall, 12% soil moisture; one large, in bloom, growing in Experimental Garden out of doors, soil moisture 12% before rain.

FIG. 18. Comparison of cultivated and wild oats, changes in light as indicated by symbols at bottom of graph; two sets of plants: cultivated oats, large, about 70 days old, 24.5% soil moisture; wild oats, large, transplanted from field to greenhouse 35 days earlier, 15–20% soil moisture.

THE ORIGIN AND NATURE OF THE MUCILAGE IN THE CACTI AND IN CERTAIN OTHER PLANTS*

Francis E. Lloyd

The increasing interest in the rôle of emulsoids in the economy of the plant, especially in that of the growth processes, has indicated the necessity of re-examining them from the more recently taken point of view exemplified in the work of Borowikoff, Long, MacDougal, Spoehr, and others.[1] More specifically regarded, the question of the relation of alterations in the volumes of emulsoids due to changes in the acid and salt content of the tissues, and the effect of these alterations on the volume of the plant body is one of paramount importance, and is particularly to the fore at the moment.[2] In a good many plants the presence of very considerable quantities of mucilages and "gums" has of course long been a matter of common knowledge and has been much investigated, chiefly from the point of view of the histologist and pharmacologist[3] with only side glances at their physiological contacts, leaving much to be said from the present point of view. The following account is confined principally to the mucilage of Opuntia *in situ*, having regard to its origin and distribution, coupling therewith some notes on the comparable conditions found in Tilia, Malva, and *Astragalus gummifer* (tragacanth).

Distribution of Mucilage Cells

The mucilage in Opuntia originates within, and is normally confined to, definite large cells (mucilage idioplasts) scattered throughout the parenchyma, both medullary and cortical. Their absolute number is correlated with the species. Their distribution in the cortex and medulla differs also in different species. Thus, in *Opuntia* sp. (a species obtained from Dr. D. Griffiths and now in the garden at the Coastal Laboratory of the Carnegie Institution of Washington) none are found within 2.5 mm. of the epidermis of the joints, though they occur more closely thereto in the leaves. In *Opuntia Blakeana*, however, they lie very near the epidermis. For this reason it is practically possible, in the former species, to cut sections of the chlorenchyma parallel to the epidermis which when allowed to lie in water give off no mucilage. There is observable no mucilage identical with that of the mucilage idioplasts in any other cells, though it is not at the moment denied that there may be a very small amount of hydrophile colloid in the vacuoles of the ordinary parenchyma cells.

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* A subvention from the Cooper Fund for Medical Research of McGill University is hereby acknowledged.

[1] For citations see MacDougal and Spoehr, 1917.

[2] MacDougal and Spoehr (*l. c.*); Lloyd, 1917, 1918*a*.

[3] See Tschirch, 1884.

The number of mucilage cells in the cortex may be smaller per unit volume of tissue than in the medulla, but on the other hand there is frequently realized a condition in which both cortex and medulla are crowded with them. In a joint of *Opuntia susquehannensis* about 3.5 cm. long, and which, though small, was shrunken as if it had been long deprived of water, the mucilage cells were so large and numerous as to occupy much more than half the total volume of the whole. They were moreover crowded upon each other to such an extent as to approximate a lacunar condition such as Walliczek (1893) described for Tilia. Trécul (1875) believed this to be true also for cacti, etc., but in view of the peculiar difficulties of observation a reasonable question of fact may be permitted.

Place and Time of Origin

Though occurring within both medulla and cortex, the mucilage cells arise first in the medulla and later in the cortex. The earliest may be found in the medulla directly beneath the growing point, while in the cortex the youngest readily recognizable as passing into the more obvious condition of a definitive mucilage cell could be found only as far as 4 mm. from the apex, in the Opuntia above mentioned. Relatively few, however, originate within the actively expanding region at the apex of a young joint. They arise rather during the whole period of growth in all regions of the enlarging joint, young ones being found even toward the base of a joint several centimeters long. They are therefore secondary in their origin, and before they assume their special character are indistinguishable from the surrounding cells, whether of cortex or medulla. It is a legitimate speculation that the numbers of mucilage cells may be modifiable under various environmental conditions.[4] In *Carnegiea gigantea* the mucilage cells are not to be found in the palisade tissues and are considerably fewer in number than in Opuntia. They are absent from the younger tissues, none being found by me in a small individual 45 cm. tall, except below the level of *ca.* 30 cm. from the apex.

Mode of Origin, Chemical Structure and Inclusions

The mucilage cells as such are at first recognizable only by their size. When once differentiable, one notes that the nucleus and nucleolus enlarge to an enormous size. At first parietal in position, the nucleus usually becomes central. The protoplasm also increases in amount both absolutely and relatively, and the nucleus becomes suspended in many thick strands. Chloroplasts and starch grains are usually present, and a large stellate cluster of calcium oxalate crystals is frequently, though not invariably, to be seen.

The wall is at first indistinguishable from the walls of surrounding cells, but when considerable size has been gained it becomes somewhat thickened.

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[4] According to Tschirch (*fide* Walliczek, 1893, p. 274), the mucilage content of the marshmallow is greater when the plant is grown in dry soil.

So long as the cell has not passed beyond the condition thus far described, the addition of water to fresh or alcohol-fixed material does not affect its internal topography, whereas when the secretion of mucilage has been begun this is not the case. Assuming however the contrary, that mucilage has already appeared, and providing that the cell is still immature, the imbibition of water from the surrounding *milieu* by the mucilage causes a displacement of the protoplast from the cell wall more or less complete.[5] This is explained by the circumstance that material capable of a high degree of hydration now occupies the inner surface of the cell wall. It can now be shown that an inner zone of this wall, approximately half as thick as the whole, is in a more hydrated condition than the remaining cell-walls because it *gives a deeper blue coloration with iodine.* The inner face of this zone is not optically definable but fades into a colorless substance, the mucilage. The amount of this present is indicated by the amount of displacement of the protoplast from the wall. Usually the protoplasm will hold to the wall at several points, especially where pits occur, and when the mucilage becomes more abundant its swelling results in the somewhat bizarre appearance of an entire protoplast compressed at the middle of the cell and connected by strands of protopasm with the wall at several or many points. The larger conspicuous strands have their distal place of attachment to the cell wall at or very near the middle points of the areas of contact of the contingent parenchyma cells. This appears clearly to indicate that the reason for adherence is the presence of the intercellular connections at these points, which are marked by wide, shallow pits.

When the amount of mucilage arrives at or near to the maximum, the imbibition of water permits its hydration to such an extent that the protoplast becomes crowded into an irregular echinate mass, the radiating protoplasmic processes being either detached from the wall or variously torn asunder. The mucilage itself is now seen, but with some difficulty, to be laminated, the zonation being parallel to the cell wall but with curvatures toward the pits. This zonation, seen in *Opuntia elatior* by Cramer (*fide* Wigand, 1863, p. 149), is due to varying degrees of hydration (Walliczek, 1893) as appears from the fact that, when dehydrated with alcohol, internal syneresis occurs much more extensively in the more hydrated zones, which are then discoverable to the eye as zones of small spherical cavities of various sizes, but all minute. Such syneretic cavities may, however, be quite large, depending, in part at least, on the rate of dehydration, and probably also on the degree of hydration of the mucilage as a whole. The lamination is generally observable before or during the course of swelling, and is much more evident in *Carnegiea gigantea.* In this form the mucilage swells more slowly, and the loss of the marked lamination during increasing hydration may be readily followed on the addition of water. The lamination in tragacanth, seen by Kützing (*fide* Wigand, 1863), is still more evident, and is lost only very slowly at ordinary temperatures, while that of Sterculia appears to be still more resistant (Maiden, *fide* Tschirch, Lehrbuch, p. 403, vol. 2, pt. 1).

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[5] In order to form a critical judgment of the condition of the mucilage cells, fresh sections must be examined without the addition of water, as should also fresh and alcohol-dehydrated material with added water.

If mucilage cells in this condition are cut open in the making of a section, the mucilage swells enormously on the addition of water, oozes out from the cell cavity and, carrying the protoplasm and its inclusions with it, forms a rope. If pieces of tissue are placed in water, they gradually become translucent. This is due to the expulsion of air from the intercullular spaces, resulting partly at any rate from the bursting *in situ* of the mucilage cells. This fact may be demonstrated by examining sections of a piece of tissue which have lain in water, the sections being dehydrated and examined in alcohol. In the case of the medulla of a frond several centimeters thick, in which the elongation of the cells had taken place in a direction normal to the surface of the frond, it was found that the bursting of the mucilage cells had taken place in this direction. This, it was evident, was due to the mechanical conditions offered by the web of cell walls and the mutual pressures of the cells. Whether such bursting occurs within the plant in consequence of local disturbances, resulting in the extrusion of mucilage into the intercellular spaces, and possibly filling lacunae (schizogenous or lysigenous, or due merely to tearing) within the tissues, is not proved, though it is rather to be expected, especially when the mucilage cells are large, numerous, and mutually contingent. It may be noted in passing that in some species of Opuntia and in other genera (*e. g.*, Ariocarpus) there are lysigenous canals or lacunae filled with a gummy secretion, but of a different nature from that being here considered.

Effect of Anaesthetics

Dr. H. A. Spoehr pointed out to me that an abundant oozing of mucilage takes place on treatment of tissue with choloroform, ether, etc. I offered the explanation that the immediate effect of the reagent was to asphyxiate the parenchyma cells by which the mucilage cells are surrounded, upon which they give up their water into the intercellular spaces, making it possible to hydrate the mucilage cells. This was verified as follows.

A section was placed without the addition of any medium on a cover glass and inverted over vapor of ether in a small glass cell. In the course of a minute, the air in the intercellular spaces began to be expelled by water escaping from the parenchyma cells. It could then be clearly seen that the mucilage cells became more hydrated, as was proved to the eye by the further displacement of the protoplast. Radial strands reaching to the cell wall could be observed in the breaking, and the whole mass of protoplasm to be further crowded toward the middle of the cell. In some instances the cell walls were broken, and the mucilage could then be seen oozing out therefrom through circular perforations, recalling the similar behavior in the tannin idioplasts of the persimmon (Lloyd, 1911). In the course of time the parenchyma cells completely collapsed, and the mucilage had then reached its maximum hydration permissible under the circumstances. The preparation was now stained in alcoholic safranin and the few mucilage cells remaining unbroken were stained. On being placed in water nearly all of these subsequently burst under microscopic observation.

Inclusions in Mucilage Cells

Starch is generally found within the protoplast *sensu stricto*, in amounts usually correlated with the amount found in the neighboring cells, but sometimes in less quantity.[6] It is perhaps unexpected to find that in a much shrunken frond of *Opuntia susquehannensis*, already referred to, the old and fully hydrated mucilage cells contained a very large amount of starch, as did the remaining parenchyma. No evidence of an inverse quantitative relation between the amount of starch present and the extent of mucilage secretion could be observed. This starch, it would seem wholly probable, was laid down after the mucilage had been secreted. This view suggests the question of the physiological condition of the mucilage cells after the amount of mucilage is sufficient to cause displacement of the protoplast from its usual and conceptually normal position, namely, against the cellulose wall. Specifically, does the protoplast become moribund and eventually die when compressed within the swollen mucilaginous mass? Without attempting at the moment to answer this question, it may be pointed out as bearing on it, that the size of the mucilage cell does not appear to remain fixed after the amount of mucilage has become sufficient to press the protoplast into a relatively small compass within its interior. It is certain, at any rate, that there is no disappearance of mucilage cells during the developmental phase of the frond, and it is similarly certain that the size of these cells in the mature tissue is much greater than in young, *quasi* embryonic material. Thus, in a frond some centimeters long, the mature mucilage cells near the growing tip measured 0.15 mm. in diameter. Toward the middle of the frond they measured fully 0.5 mm. in diameter, a gradual increase in size being observable as the eye receded from the growing apex. In view of the possible secretion of starch above mentioned, it seems possible that in spite of the crowding of the protoplast by the hydrated mucilage, it remains alive, and that the cell grows. In this event, the pressure on the cell wall which causes stretching is dominantly the imbibition pressure of the mucilage.

General Discussion

A review of the literature pertaining to the matter under present treatment shows clearly that the essential features of the topography of the mucilage cells not only in the cacti but also in the mallows, Tilia, Sterculia, tragacanth (*Astragalus gummifer* Labill), etc., have been comprehended. Kützing (through Wigand, 1863) as early as 1851 saw the lamination in tragacanth, but had an entirely incorrect idea of the origin of the mucilage cells, regarding them as a fungus. Mohl in 1857 (also through Wigand) explained the appearance of gum tragacanth as due to the centripetal deorganization of the cell membranes and their change into the gum, a view which certain later observers (Karsten, Schleiden, Wigand) adopted. Cramer in 1855 saw the lamination of the mucilage cells in the cacti. Wigand (1863, p. 149) described the collapsed protoplasmic utricle as a more or less evident trace of an ill-defined cavity, with radiating arms ("radiations") penetrating the mucilage-content in a manner analogous to pore-canals, without properly apprehending the significance of these details. Walliczek (1893) correctly described in a topographical sense the structure of the cell as a whole, but erred, as I think, with Longo (1896) in regarding the mucilage in Epiphyllum, etc., as granular, the granules (so regarded by Walliczek) being merely cavities (Longo) due to dehydration by alcohol, in which medium Walliczek examined his material. His description of the mucilage cells in *Opuntia Tuna* appears to be incorrect, since "mucilage strands stretched in an irregular network through the lumen" do not occur. It would seem that he misinterpreted one of the bizarre conditions caused by rapid hydration in which there are numerous delicate and meshed strands of protoplasm passing out radially through the mucilage to the cell wall. This appearance I have often seen, and is caused by the pinching of the protoplasm by mutually appressed masses of mucilage. Under such conditions the protoplasm is squeezed into lacunated layers, thus producing the meshed appearance described by Walliczek.

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[6] It is also found in the mucilage ("gum") cells of tragacanth, as noted by earlier observers.

Concerning the mode of origin of the mucilage there are diametrically opposed views, namely: (*a*) that the mucilage is secreted within the protoplasm (Lauterbach, 1889, *fide* Walliczek, p. 267) or in extreme form that the mucilage cells have a "plasma gommeux, qui vie et végète à la manière du plasma des cellules ordinaires" (Trécul, 1862, and restated in 1875); and (*b*) that the mucilage is some form or product of the cell wall. The latter view appears in different forms.

Wigand thought that the mucilage arises by a deorganization of an already present secondarily much thickened cell wall. De Bary (1884, p. 144), following Wigand (whom he cites), and apparently depending on his descriptions, regarded the mass of mucilage in the cells of mallows, cacti, and laurels as having the "structure of a very thick, abundantly and delicately stratified cell wall," and that it is . . . nothing more than a cell wall *which has thickened* [italics mine] strongly at the expense of the central cavity."

Walliczek (p. 268) thought that the mucilage is *laid down on the primary membrane as a secondary thickening*, in the formation of which the primary cell wall takes no active part, saying specifically that the plasma lays down the secondary wall (mucilage) *on its outer external surface.* He believes that in so stating the case, he agrees with de Bary and not with Wigand.[7] It has however been shown in the previous pages of this paper that the inner portion of the original cell wall is altered into a hydrocellulose at the time when mucilage begins to appear. The mucilage arises therefore by hydrolysis of the original cell wall which shows no striking or excessive secondary thickening, and not by deposition by the plasma on this wall of additional new material, or by the alteration of a thick secondary cell wall, whether laid down as cellulose or as bassorin. Neither Walliczek's view, nor that stated by de Bary, is therefore correct.

This account applies equally to the mallows, cacti, Tilia and tragacanth.[8] The last named I have been able to study only from a fragment of stem opportunely included in a fragment of the gum, and from the gum itself. In agreement with Mohl and Wigand, I found the lamination of the mucilage, and the included starch. I found also fragments of the original cell walls, both of mucilage cells and of non-mucilaginous parenchyma cells. The walls of the mucilage cells bear evidence of extensive hydrolysis, as they are incomplete and show thinned-out edges, while the others show a tearing effect. The protoplasmic utricula with included plastids and starch grains are also very easily identifiable. The original cell walls in the gum are usually very thin and only partially present,[9] and it would seem that in addition to hydrolysis of the cell-wall, that of the middle lamellae must also have taken place in order to bring about the result seen in gum tragacanth, especially in view of the manner of its exudation. This would seem to explain the large amount of "composé pectique," in part pectose, which analyses of gum tragacanth have furnished (Giraud, through Tschirch, p. 399). Bassorin is described as insoluble in water, and is regarded as furnishing only a mechanical suspension as compared with the mucilages of cacti, etc. The distinction is hardly justified. It is true that tragacanth produces an imperfect solvation, the degree depending on temperature, etc., somewhat as in the case of agar-agar, and that in any event there is a lack of homogeneity in the dispersion as compared with one of cactus mucilage. Somewhat the same sort of difference is found on comparing the mucilage of Tilia or Malva with that of Opuntia, the latter yielding a much more viscous product. Carnegiea yields a mucilage of low viscosity, quite as low as that of Tilia. Indeed I find like differences between different species of Opuntia. Doubtless a refined technic would discover chemical differences between these various mucilages. Here it is only to say that the distinction between a "gum" and a mucilage is not, at the present, one which corresponds with the manner of origin of these substances. It is pertinent in this connection to remark that Tschirch's organological classification is in a sense inadequate, as it brings into association mucilages of distinctly different kinds: *e. g.*, salep is intercalated between the mallows and tragacanth.

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[7] It is hardly profitable to consider Wigand's views too seriously, since he evidently confused the cytology of the mucilage cells of salep (*Orchis* sp.) with that of the mucilage cells of cacti and mallows (p. 149). Indeed his view quoted above was based on the raphide cells of salep and immediately applied to the cacti—an obviously impossible comparison.

[8] Whether the "gum" of *Sterculia* sp. is to be included with these is doubtful. But the "bassora-gum" studied by Wigand (*l. c.*) showed without any doubt that, whatever its origin, unknown to Wigand, it has the same character as tragacanth. Sterculia gum is said not to show lamination (Maiden, *fide* Tschirch, vol. 2, pt. 1, p. 400).

[9] Just what the thickness of the original walls is in tragacanth I am unable to say. Judging however from the illustrations available (Tschirch), they show no evidence of marked thickening previous to the arising of the mucilage.

CHEMICAL AND STAINING REACTIONS OF THE MUCILAGE

The determination of the chemical composition of the mucilage is obviously a problem within the field of biochemistry, and it is, therefore, not my purpose to pass beyond the limits set by the methods I have used.

It has been shown that previous to the occurrence of mucilage, the inner zone of the cell wall gives the reaction of hydrocellulose. The mucilage itself gives no indication of this origin, as with iodine and sulphuric acid it colors only yellow or brownish. It is therefore a cellulose-mucilage if regarded from the point of view of its origin.

It is hydrolyzable by chromic acid, though very considerably more resistant to this reagent than the middle lamella. The tissues may be immersed for several hours in 10% chromic acid, whereby the mucilage cells are set free in their entirety, and, after washing, may be preserved indefinitely in water, and in this condition afford practically unaltered pictures. If the action of the reagent is more prolonged, more or less swelling occurs and consequent rupture beyond the confines of the cell walls. Ultimate complete hydrolysis follows still longer treatment, especially at higher temperatures.

The mucilage is hydrolyzed also by sulphuric, nitric, and hydrochloric acids.

It submits slowly to the digestive action of organisms. The time occupied in materially reducing the viscosity of a solution sufficiently for it to be recognizable to the eye was about six weeks. At the end of eight to ten weeks the viscosity had been lowered to that of water, or nearly so. A second lot, having an initial viscosity several times greater than the above, still shows after six months a considerable viscosity, but much nearer to that of water than to its initial viscosity. During the process a series of odors has been produced, some of which were undoubtedly due to protein putrefaction. The organisms involved are not yet determined.

STAINING

No attempt has been made to exhaust the possibilities of staining the mucilage cells in the ordinary sense.[10] For the purpose of demonstrating their distribution and behavior during swelling safranin in alcoholic solution was found very good. Thick sections are first stained and are then allowed to hydrate under the microscope. The total enlargement of the mucilage cells, due to the swelling of the imprisoned mucilage, is shown in the strains on the walls of the neighboring cells. This affords a picture of what may occur in the growing plant when under altered conditions of acidity the volume of the mucilage cells is altered.

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[10] For the ordinary methods see Strasburger-Koernicke's Botanisches Practicum; Tunmann; Tschirch.

Neutral red. If living sections are placed in a very weakly acidified solution (I used acetic acid) of low concentration of the stain, the mucilage *in situ* will slowly take up the stain. Pronounced results may not be expected in less than twenty-four hours. The cell walls also stain, their color being a deeper red as compared with the rose pink of the mucilage, a difference doubtless referable to the degrees of dispersion of the colloidal systems. The annulae of the vessels also stain pink. In a strong solution of the stain the strong coloration of walls and protoplasm and the deep staining of the mucilage itself make observation difficult.

In a slightly alkaline (to neutral red) solution the mucilage is not stained, or at length very slightly, although the included protoplast becomes deeply stained. The cell sap (in living sections) becomes loaded with the stain.

Ruthenium red is taken up vigorously by the mucilage, as also by the cell walls and protoplasm.[11] The stain has a dehydrating effect ont he mucilage, which, when in high enough concentration, is sufficient to prevent swelling.

The above mentioned behavior of mucilage toward neutral red in acid and alkaline solution, and the dehydrating effect of ruthenium red, coupled with other frequent observations of my own which need not be detailed, led me to inquire more particularly into the relation of the mucilage as an emulsion colloid to dyes in general. It may be recalled that I showed some time ago (1911) that tannin is adsorbed by a cellulose-like body when coagulated, and only weakly so when not coagulated. This appears as a parallel behavior to that of cactus and other mucilage toward neutral red. At all events it was evident that the adsorption of a stain by the mucilage is related to the degree of hydration, and on attempting to investigate this relation it was further determined that certain dyes themselves alter the dispersive relations of the mucilage. *E. g.*, it was found that ruthenium red *forms membranes on the surface of a mass of mucilage* (Opuntia, Tilia, etc.) as tannin does on a hydrated albumin. On mixing dyes with mucilage it developed that certain of them *gradually lower the viscosity of the mucilage* till it approaches closely that of water, and that the dyes which are most effective are those which are most strongly adsorbed. The emulsion-colloidal properties are thus quite altered, and the complex takes on the character of a suspension (Lloyd, 1918*b*). By means of alcohol, further dehydration followed by precipitation occurs and the amount of adsorbed stain is reduced, when the mucilage may be recovered as such but with slightly altered physical properties, since it no longer swells indefinitely in water. The following stains have been investigated as to this behavior. They are mentioned in series, beginning with the most vigorously adsorbed and therefore the most active in reducing viscosity. The material was from *Opuntia Blakeana.* Ruthenium red > neutral red > Bismarck brown = gentian violet = methylene blue > safranin > methyl green > erythrosin. The viscosity was unaltered after nine days by fuchsin, methyl blue, coralline, orange G, methyl orange. For the concentrations used, an observable lowering of viscosity followed after twenty-four to forty-eight hours in the case of the most vigorously adsorbed stains. A brief examination of the mucilage of *Ulmus fulva* and of Linum (seed-coat) has indicated that they behave similarly toward ruthenium red and neutral red. Further investigation is in progress.

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[11] It is rather usual (*e. g.*, Ishikawa, 1918) to quote this reagent as staining the pectic substances, allowing the inference to be drawn that it is specific in this regard. This is, however, not in any sense true, as I have previously observed (1916, p. 219.) Further on this, however, beyond.

Conclusions

1. Mucilage in the cacti, mallows, and tragacanth arises within specialized parenchyma cells by hydrolysis of the cellulose wall, *which is not secondarily thickened.* The first visibly demonstrable change is from cellulose to a hydrocellulose; from this the mucilage arises. As this hydrates, it swells and compresses the protoplasm toward the middle of the cell. The protoplasm remains attached more or less to the pits (where little or no hydrolysis of the wall appears to occur), giving rise to radiating strands mimicking the strands within the protoplast extending from the nucleus to the wall-layer.

The mucilage shows lamination which is determined by water content. It is quite possibly predetermined by the apposed layers of cellulose in the original cell wall. The lamination has evidently led certain previous observers to the idea that the mucilage arises as a secondary thickening in the structural sense.

The mucilage is neither laid down as a secondary layer, nor is it secreted within the protoplast, nor yet is it a secretion thrown out as mucilage from the outer surface of the protoplasm.[12]

In tragacanth it appears that the hydrolysis of the cell walls is more extensive than in such forms as Opuntia, Tilia, the mallows, and is at the same time accompanied by digestion of the middle lamella.

2. The mucilage adsorbs certain dyes with great vigor, others with lesser and different degrees of vigor, and still others not at all.

The viscosity of the mucilage is lowered by those dyes which are adsorbed, at a rate and to an extent in direct relation to the degree of adsorption.

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[12] See summary in Tunmann (1913).

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THE INFLUENCE OF PHOSPHATES ON THE ACTION OF ALPHA-CROTONIC ACID ON PLANTS

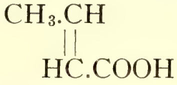
J. J. Skinner and F. R. Reid

Introduction

The physiological effects of α-crotonic acid on plants and the action of nutrient salts in counteracting these effects have been studied in this laboratory, and are reported in this paper. The investigation comprised a study of the effects of the compound on plants grown in pure distilled water and in nutrient solutions composed of phosphate salts together with sodium nitrate and potassium sulphate. Some preliminary tests of the compound were made in soil in pots, but this phase of the subject was not gone into exhaustively and the results will not be given here.

α-Crotonic acid was isolated in this laboratory,[1] first from a sample of Susquehanna fine sandy loam soil from Texas. The soil was taken from an infertile spot in a field near Marshall, Texas. The infertile spots, which were devoid of all vegetation, had been observed for several years in this locality; the areas of these spots were gradually increasing.

The soil in this district is described as a fine sandy loam, from 8 to 18 inches deep, with an average depth of about 14 inches.[2] The sub-soil is a stiff clay of a red color or red mottled with yellow and gradually extending to a depth of several feet. The drainage is very poor because of the impervious nature of the sub-soil. The soil is deficient in lime or other basic material and is very poorly drained. It has been found to have a high reducing power and a low oxidizing power. The conditions for the accumulation of organic acids seem favorable.

α-Crotonic acid 

$$\begin{array}{c}CH_3.CH\\ \|\\ HC.COOH\end{array}$$

is produced from allyl cyanide, which is a constituent of mustard oil, and it has been isolated from pyroligneous acid obtained by the dry distillation of wood.[3]

Crotonic acid is shown to be very harmful to plants in pure water and in nutrient culture solutions. With distilled water the compound in concentrations as high as 200 parts per million killed wheat plants; in concentrations of 10 parts per million growth of tops was reduced 10 percent, but the roots were not affected; in 25 parts per million growth was materially reduced, green weight of tops was reduced 27 percent, while the growth of roots was checked; in concentrations of 100 parts per million growth was reduced 33 percent. The roots of the plants in this concentration made only a small growth; they were short and stunted. The harmfulness was still very marked in these high concentrations when calcium carbonate was added to the solutions. In weaker concentrations the effects were not so severe in the presence of lime.

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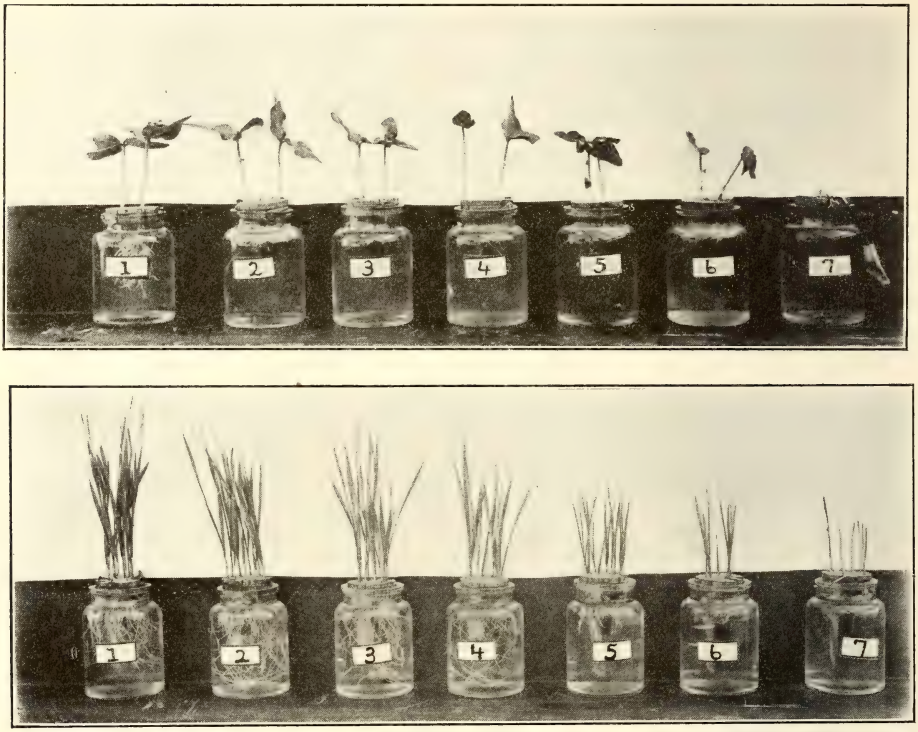


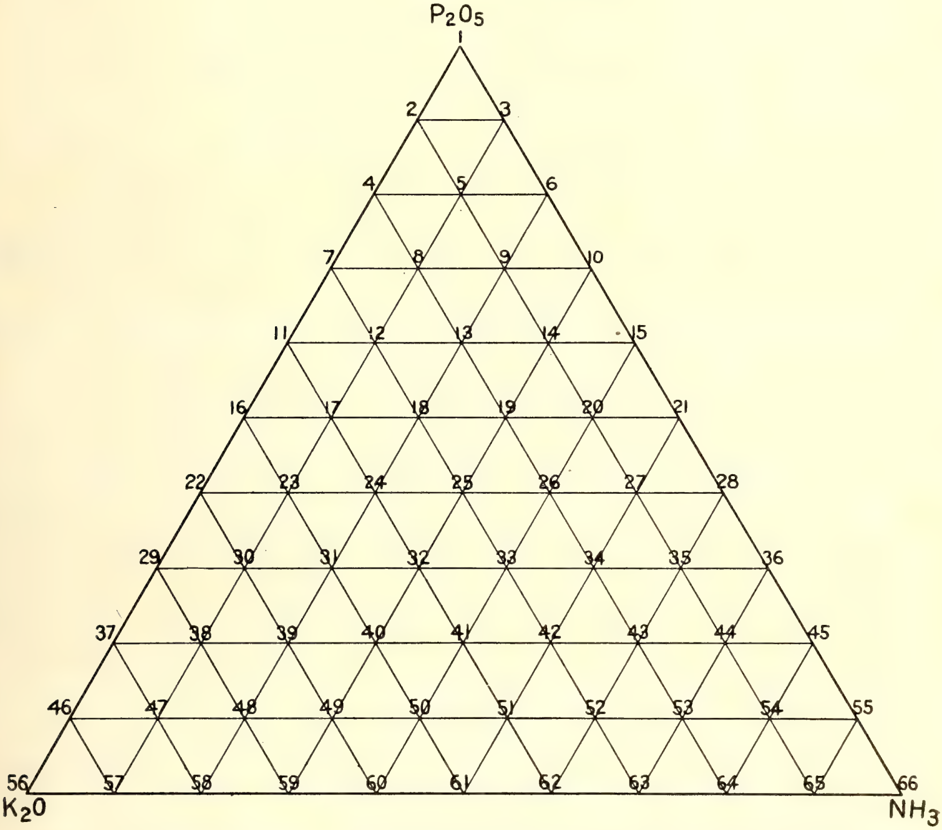
FIG. 1. Effect of α-crotonic acid on cowpeas and wheat.

(1) Distilled water, (2) α-crotonic acid 10 parts per million, (3) 25 parts per million, (4) 50 parts per million, (5) 100 parts per million, (6) 200 parts per million, (7) 500 parts per million.

In figure 1 is shown the effect of various concentrations of the crotonic acid on wheat and cowpea plants grown in distilled water. The striking effect of the organic acid is here apparent.

NUTRIENT CULTURE SOLUTIONS

An extensive study was made, growing wheat plants in nutrient solutions of calcium acid phosphate, sodium nitrate, and potassium sulphate, without and with crotonic acid. The familiar triangle system which has been used widely in a study of nutrient solutions and organic compounds was used in these experiments.[4] The essential constituents, P_2O_5, NH_3, and K_2O, of the three salts are present to the extent of 80 parts per million, but the composition varies. The details of the system, which are familiar to scientific investigators, are given in the publications cited, and only the general features of the triangle will be explained here.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NH3

FIG. 2. Triangular diagram, with the points representing the 66 culture solutions numbered.

The triangular diagram shown in figure 2 is used as a guide. In this diagram the apices of the triangle, numbers 1, 56, and 66, are the cultures which contain only the single salts, calcium acid phosphate, sodium nitrate, and potassium sulphate respectively, each containing 80 parts per million of P_2O_5, NH_3, or K_2O respectively. The line of cultures from 1 to 66 contains mixtures of P_2O_5 and NH_3 in 10 percent differences; the line of cultures from 1 to 56 contains mixtures of P_2O_5 and K_2O in 10 percent differences; the line of cultures from 56 to 66 contains mixtures of K_2O and NH_3. The cultures in the interior of the triangle contain mixtures of all three constituents, differing in 10 percent stages one from the other, the composition depending upon its position in the triangle; those nearer the P_2O_5 apex consisting chiefly of phosphate salt, those nearer the NH_3 apex chiefly of nitrate salt, and those nearer the K_2O apex chiefly of potash salt. For a more detailed explanation of the scheme and principles involved, the reader is referred to the earlier papers.

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[4] Schreiner, O., and Skinner, J. J. Some effects of a harmful organic soil constituent. U. S. Dept. Agr. Bur. Soils. Bull. 70, 1910. Ratio of phosphate, nitrate and potassium on absorption and growth. Bot. Gaz. **50**: 1. 1910.

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In table 1 is given the composition of the solution represented by each of the 66 points in the diagram.

TABLE 1

The composition of the sixty-six nutrient solutions, the constituents P_2O_5, NH_3, and K_2O varying in 10 percent stages

Point No.	P.p.m. Present in Solution			Point No.	P.p.m. Present in Solution			Point No.	P.p.m. Present in Solution		
	P_2O_5	NH_3	K_2O		P_2O_5	NH_3	K_2O		P_2O_5	NH_3	K_2O
1	80	0	0	23	32	8	40	45	16	64	0
2	72	0	8	24	32	16	32	46	8	0	72
3	72	8	0	25	32	24	24	47	8	8	64
4	64	0	16	26	32	32	16	48	8	16	56
5	64	8	8	27	32	40	8	49	8	24	48
6	64	16	0	28	32	48	0	50	8	32	40
7	56	0	24	29	24	0	56	51	8	40	32
8	56	8	16	30	24	8	48	52	8	48	24
9	56	16	8	31	24	16	40	53	8	56	16
10	56	24	0	32	24	24	32	54	8	64	8
11	48	0	32	33	24	32	24	55	8	72	0
12	48	8	24	34	24	40	16	56	0	0	80
13	48	16	16	35	24	48	8	57	0	8	72
14	48	24	8	36	24	56	0	58	0	16	64
15	48	32	0	37	16	0	64	59	0	24	56
16	40	0	40	38	16	8	56	60	0	32	48
17	40	8	32	39	16	16	48	61	0	40	40
18	40	16	24	40	16	24	40	62	0	48	32
19	40	24	16	41	16	32	32	63	0	56	24
20	40	32	8	42	16	40	24	64	0	64	16
21	40	40	0	43	16	48	16	65	0	72	8
22	32	0	48	44	16	56	8	66	0	80	0

Two sets of these 66 solutions were prepared, one set containing the crotonic acid. The culture solutions were contained in wide-mouth bottles and 10 wheat plants were grown in each culture after the manner described in the papers cited. The solutions were changed every three days, four such changes being made in each experiment and analyzed immediately after each change for nitrates, but the phosphates and potash were determined on a composite of the four changes. The green weight of the plants was determined at the termination of the experiment.

In the first experiment using the two triangle sets of solutions, crotonic acid was added to one set in amounts of 50 parts per million, the other being used as a control or check set. The experiment ran from December 6 to December 18, 1916. The green weight for each culture is given in the two charts in figure 3. The crotonic acid reduced growth very much as shown by comparing the green weight figures. The roots of the plants grown in crotonic acid solutions were also severely retarded in their development. The green weight of the 66 cultures in the normal set was 143.6 grams, and that of the crotonic acid set 69.16 grams. The effect of the crotonic acid was to depress the green weight 52 percent.

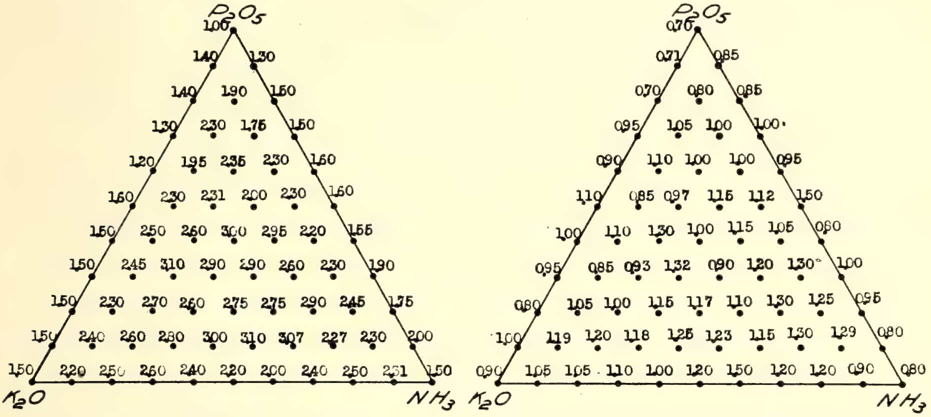

FIG. 3. Green weight in grams of wheat plants in nutrient solutions containing varying ratios of phosphate, nitrate, and potash, the source of P_2O_5 being calcium acid phosphate. (1) Without crotonic acid, and (2) with 50 p.p.m. crotonic acid.

The crotonic acid had a more severe effect in some of the solutions than

TABLE 2

Showing the influence of phosphate in overcoming the harmful effect of α-crotonic acid. Green weight of wheat plants in nutrient solutions composed of $CaH_4(PO_4)_2$, $NaNO_3$, and K_2SO_4. Cultures arranged according to content of P_2O_5.

Culture No. (See Fig 2)	Parts per Million of P_2O_5 in Nutrient Solution	Average Green Weight of Culture		Percentage Decrease Due to Crotonic Acid
		Without Crotonic Acid	With 50 P.p.m. Crotonic Acid	
		grams	*grams*	
1	80	1.00	.70	30
2–3	72	1.35	.78	42
4–6	64	1.60	.77	51
7–10	56	1.74	.80	53
11–15	48	1.88	.99	47
16–21	40	2.02	1.11	45
22–28	32	2.73	1.06	55
29–36	24	2.46	1.05	57
37–45	16	2.41	1.07	56
46–55	8	2.50	1.15	54
56–66	0	2.20	1.03	55

in others. The growth is more nearly normal in the solutions high in phosphate, but is much depressed in the solutions in the lower part of the triangle, that is, in those low in phosphate.

In table 2 are given the green weights of the various series of cultures containing the same amount of phosphate, that is, the series along any one of the horizontal lines in figure 2.

The last column of the table gives the percentage decrease caused by the crotonic acid. In those cultures which contained 80 parts per million P_2O_5, growth was reduced 30 percent, and in the cultures containing 72 parts per million P_2O_5, 42 percent, while in the cultures containing 32 parts per million of P_2O_5 and less, growth was reduced as much as 55 percent.

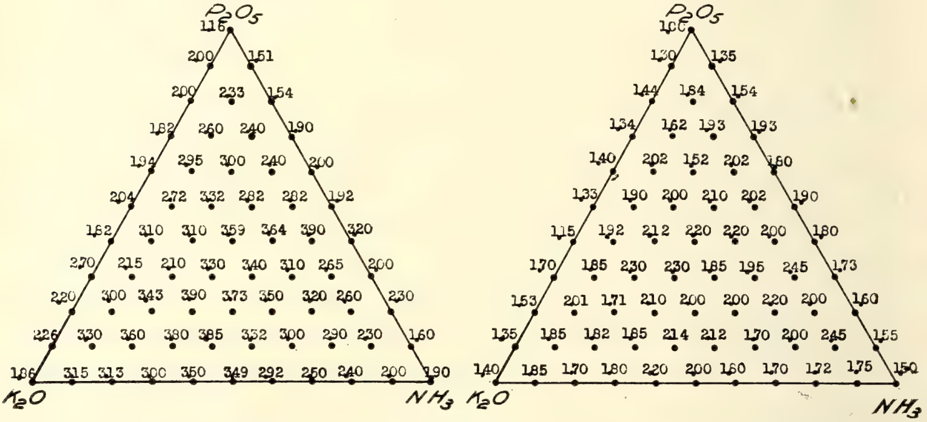

FIG. 4. Green weight in grams of wheat plants in nutrient solutions containing varying ratios of phosphate, nitrate, and potash, the source of P_2O_5 being calcium acid phosphate. (1) Without crotonic acid, and (2) with 25 p.p.m. crotonic acid.

In another experiment similar to the one just described, the crotonic acid was used in amounts of 25 parts per million. In all other details the two experiments were similar. This was conducted February 14–26, 1917. The green weights of the two sets of plants are given in the charts in figure 4. There is a difference in growth of 35 percent. The crotonic acid was markedly harmful in the nutrient solutions even with this concentration.

By an examination of table 3, where the cultures are arranged according to their P_2O_5 content, it is again seen that calcium acid phosphate was very effective in overcoming the crotonic acid.

The lessened toxicity of crotonic acid in solutions high in phosphate is also shown when the results of the experiment are grouped in such a way as to obtain all cultures containing 50 percent and over of any one of the three constituents P_2O_5, NH_3, and K_2O. This is accomplished by taking the cultures contained in the smaller triangle formed at each angle of the larger one shown in figure 2, that is, the cultures contained within the triangles: 1–16–21; 21–61–66; and 16–56–61 respectively.

TABLE 3

Showing the influence of phosphate in overcoming the harmful effect of α-crotonic acid. Green weight of wheat plants in nutrient solutions composed of $CaH_4(PO_4)_2$, $NaNO_3$, and K_2SO_4. Cultures arranged according to content of P_2O_5.

Culture No. (See Fig. 2)	Parts per Million of P_2O_5 in Nutrient Solution	Average Green Weight of Culture		Percentage Decrease Due to Crotonic Acid
		Without Crotonic Acid	With 25 P.p.m. Crotonic Acid	
		grams	*grams*	
1	80	1.10	1.00	9
2–3	72	1.75	1.32	25
4–6	64	1.96	1.61	18
7–10	56	2.18	1.73	20
11–15	48	2.46	1.55	25
16–21	40	2.61	1.87	28
22–28	32	3.20	1.91	40
29–36	24	2.68	2.02	26
37–45	16	3.10	1.91	40
46–55	8	2.99	1.88	38
56–66	0	2.66	1.77	34

There is a reduction in growth of only 22 percent by the crotonic acid in the phosphate end of the triangle, a reduction of 30 percent in the nitrate end of the triangle, and a reduction of 39 percent in the potash end of the tirangle.

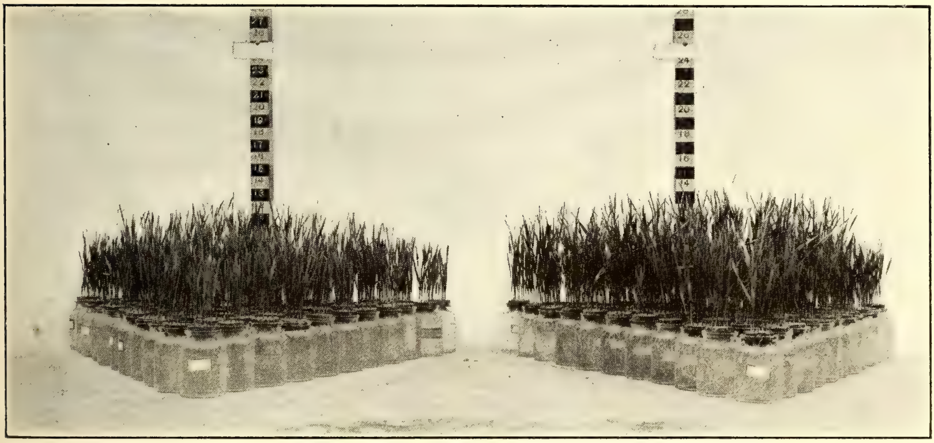

FIG. 5. Wheat plants in triangle sets of nutrient salts. (1) With 25 p.p.m. α-crotonic acid, (2) without α-crotonic acid.

The two sets of cultures are shown in figure 5, the crotonic acid set on the left and the normal set, containing nutrient salts only, on the right.

Several other experiments were planned so as to determine whether calcium or phosphate, or the salt as a whole, produced the antitoxic effect on the crotonic acid. These experiments were made by using sodium salts instead of calcium, and employing all three sodium salts of phosphoric acid, *viz.*, the mono-sodium phosphate (NaH_2PO_4) which like calcium acid phosphate is acid in reaction, the di-sodium phosphate (Na_2HPO_4) which is neutral; and the tri-sodium phosphate (Na_3PO_4) which is alkaline in reaction. In all other respects the culture solutions were the same in concentration and in composition as in the first experiment described where calcium acid phosphate was used as the source of phosphate. In each of the three following experiments, one of the sodium phosphate salts was substituted for the calcium phosphate.

EFFECT OF MONO-SODIUM PHOSPHATE

The green weight of the wheat plants in the two sets of nutrient cultures are given in the two charts of figure 6. In both of these triangle sets, mono-sodium phosphate (NaH_2PO_4) was used as the phosphate salt, while the nitrate and potash salts were the same as in the former experiment. The crotonic acid was used in a concentration of 25 parts per million. The plants as before grew for 12 days; the solutions were changed three times.

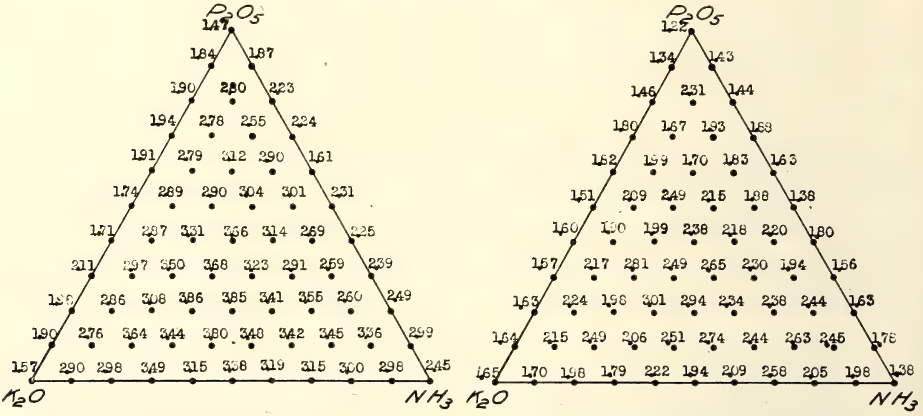

FIG. 6. Green weight in grams of wheat plants in nutrient solutions containing varying ratios of phosphate, nitrate, and potash, the source of P_2O_5 being mono-sodium phosphate. (1) Without crotonic acid, and (2) with 25 p.p.m. crotonic acid.

The harmfulness of the crotonic acid is again demonstrated in this experiment. Its relative toxicity in solutions of different composition is again shown to vary. It was again least harmful in the solutions containing the higher amounts of P_2O_5. This is shown by examining table 4.

In general the mono-sodium phosphate had somewhat the same effect as the mono-calcium phosphate; the higher the amount of phosphate in the culture solution the slighter the toxicity of the organic acid.

When the cultures are grouped, so as to bring together all those in the phosphate end of the triangle, and all those in the nitrogen end, and all those in the potash end, so as to compare each group of cultures in the normal triangle with the similar group in the crotonic acid triangle, it also becomes apparent that the phosphate has lessened the toxicity of the compound. In the 21 mainly phosphatic cultures growth was reduced 26 percent, in the 21 mainly nitrogenous, 28 percent, and in the 21 mainly potassic, 30 percent.

TABLE 4

Showing the influence of phosphate in overcoming the harmful effect of α-crotonic acid. Green weight of wheat plants in nutrient solutions composed of NaH_2PO_4, $NaNO_3$, and K_2SO_4. Cultures arranged according to content of P_2O_5

Culture No. (See Fig. 2)	Parts per Million of P_2O_5 in Nutrient Solution	Average Green Weight of Culture		Percentage Decrease Due to Crotonic Acid
		Without Crotonic Acid	With 25 P.p.m. Crotonic Acid	
		grams	*grams*	
1	80	1.47	1.22	17
2– 3	72	1.86	1.39	25
4– 6	64	2.31	1.74	25
7–10	56	2.38	1.82	24
11–15	48	2.47	1.79	27
16–21	40	2.64	1.92	27
22–28	32	2.81	2.01	28
29–36	24	2.93	2.18	26
37–45	16	3.07	2.29	25
46–55	8	3.22	2.29	29
56–66	0	2.93	1.91	35

The results with the mono-sodium phosphate are therefore similar to those with the monocalcium phosphate. This experiment seems to show that the calcium in the phosphate salt played no significant part in the observed action, since the same general action is produced by the mono-sodium phosphate.

EFFECT OF DI-SODIUM PHOSPHATE

In the experiment using di-sodium phosphate (Na_2HPO_4) the wheat seedlings grew from May 2 to May 14, 1917. The green weight results for

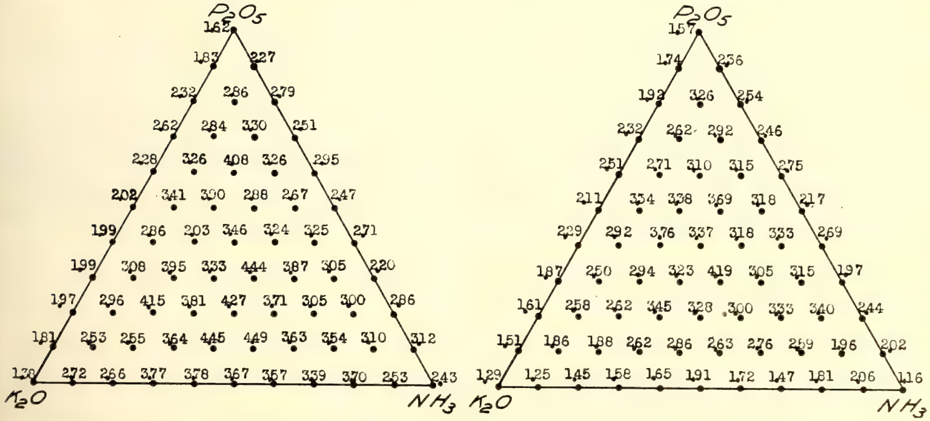

FIG. 7. Green weight in grams of wheat plants in nutrient solutions containing varying ratios of phosphate, nitrate, and potash, the source of P_2O_5 being di-sodium phosphate. (1) Without crotonic acid, and (2) with 25 p.p.m. crotonic acid.

each are given in the two charts of figure 7. In table 5 are given the average weights of the cultures arranged according to their P_2O_5 content. It will be observed that this neutral phosphate salt had a similar action in ameliorating the effects of the crotonic acid to that of the two acid phosphate salts used in the former experiments.

TABLE 5

Showing the influence of phosphate in overcoming the harmful effects of α-crotonic acid. Green weights of wheat plants in nutrient solutions composed of Na_2HPO_4, $NaNO_3$, and K_2SO_4. Cultures arranged according to content of P_2O_5.

Culture No. (See Fig. 2)	Parts per Million of P_2O_5 in Nutrient Solution	Average Green Weight of Culture		Percentage Decrease or Increase in Crotonic Acid Culture
		Without Crotonic Acid	With 25 P.p.m. Crotonic Acid	
		grams	*grams*	
1	80	1.62	1.57	− 4
2– 3	72	2.06	2.05	0
4– 6	64	2.66	2.57	− 3
7–10	56	2.82	2.58	− 8
11–15	48	3.13	2.84	−10
16–21	40	2.89	2.98	+ 3
22–28	32	2.79	3.07	+10
29–36	24	3.24	2.87	−12
37–45	16	3.31	2.86	−14
46–55	8	3.28	2.27	−30
56–66	0	3.06	1.58	−49

In the group of cultures containing no phosphate there was a reduction of 49 percent in growth due to the compound while there was scarcely any reduction in the cultures containing a large percentage of this element.

This point is again brought out when the cultures of the two triangles are arranged in groups, composed of the mainly phosphatic solutions, sub-

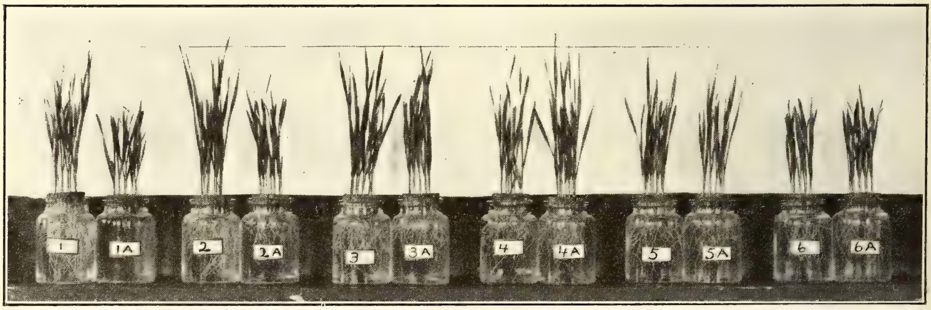

FIG. 8. Showing the effect of di-basic sodium phosphate in overcoming the harmfulness of α-crotonic acid. Bottles marked A contain 25 p.p.m. α-crotonic acid.

Nos. 1 and 1A contain no P_2O_5, 40 p.p.m. NH_3, and 40 p.p.m. K_2O;
Nos. 2 and 2A, 8 p.p.m. P_2O_5, 40 p.p.m. NH_3, and 32 p.p.m. K_2O;
Nos. 3 and 3A, 24 p.p.m. P_2O_5, 32 p.p.m. NH_3, and 24 p.p.m. K_2O;
Nos. 4 and 4A, 40 p.p.m. P_2O_5, 24 p.p.m. NH_3, and 16 p.p.m. K_2O;
Nos. 5 and 5A, 56 p.p.m. P_2O_5, 16 p.p.m. NH_3, and 8 p.p.m. K_2O;
Nos. 6 and 6A, 72 p.p.m. P_2O_5, 8 p.p.m. NH_3, and no K_2O;

triangle 1–16–21, figure 2; the mainly nitrogenous, subtriangle 21–61–66, and the mainly potassic solutions, subtriangle 56–16–61. In the 21 cultures high in phosphate growth was reduced by the crotonic acid 5 percent, in the mainly nitrogenous cultures growth was reduced 25 percent, and in the mainly potassic, 28 percent.

Twelve of the cultures from these two sets are shown in figure 8; cultures 1 and 1A contain nitrogen and potash but no phosphate. The cultures marked "A" contain 25 parts per million of crotonic acid. Here it is seen that the crotonic acid has reduced growth considerably. This is also true in cultures 2 and 2A, which contain only 8 parts per million of P_2O_5. In cultures 3 and 3A, which contain 24 parts per million P_2O_5, growth is reduced only slightly, the roots being checked more than the tops. In the other three sets of cultures, nos. 4, 5, and 6, growth of both tops and roots is about as good in the crotonic acid as in the normal solution. Culture 4 contains 40 parts per million P_2O_5; culture 5, 56, and culture 6, 72 parts per million P_2O_5.

Effect of Tri-sodium Phosphate

In the entire set of cultures in which the tri-sodium phosphate (Na_3PO_4) was used with sodium nitrate and potassium sulphate, the harmfulness of the crotonic acid was relatively less than in the former experiments. However, it is seen by examining the charts of figure 9 and table 6 that in the solutions which contained none or only a small amount of phosphate, the crotonic acid had much more effect than in solutions containing a higher amount of this element. In fact, where as much as 64 parts per million of phosphate were used growth was larger in the cultures containing crotonic acid. The effect of the organic acid was more severe in the line of cultures containing no phosphate.

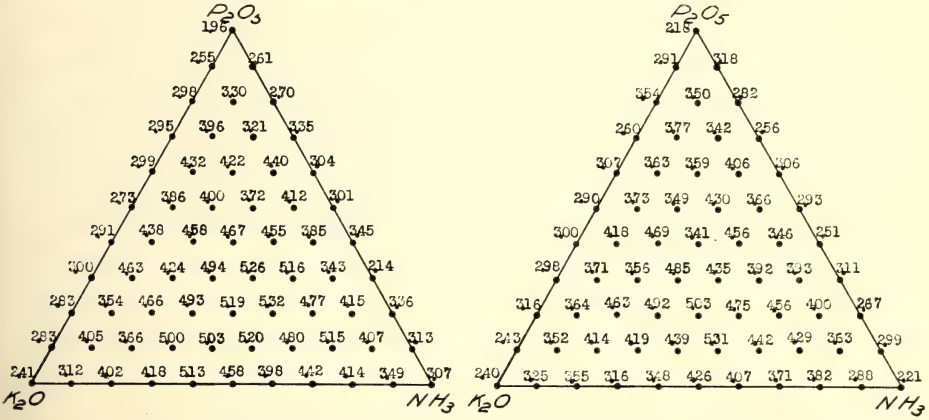

FIG. 9. Green weight in grams of wheat plants in nutrient solutions containing varying ratios of phosphate, nitrate, and potash, the source of P_2O_5 being tri-sodium phosphate. (1) Without crotonic acid, and (2) with 25 p.p.m. crotonic acid.

TABLE 6

Showing the influence of the phosphate in overcoming the harmful effect of α-crotonic acid. Green weight of wheat plants in nutrient solutions, composed of Na_3PO_4, $NaNO_3$, and K_2SO_4. Cultures arranged according to content of P_2O_5.

Cultures No. (See Fig 2)	Parts per Million P_2O_5 in Nutrient Solution	Average Green Weight of Culture		Percentage Increase or Decrease in Crotonic Acid
		Without Crotonic Acid	With 25 P.p.m. Crotonic Acid	
		grams	*grams*	
1	80	1.96	2.18	+11
2– 3	72	2.58	3.04	+18
4– 6	64	2.99	3.29	+11
7–10	56	3.37	3.09	− 9
11–15	48	3.79	3.50	− 8
16–21	40	3.57	3.50	− 2
22–28	32	4.06	3.69	− 3
29–36	24	4.10	3.80	− 7
37–45	16	4.31	4.15	− 4
46–55	8	4.29	3.93	− 9
56–66	0	3.87	3.34	−10

When the cultures are grouped, as discussed in the former set, the 21 mainly phosphate cultures were reduced in growth only 2 percent by the crotonic acid, the 21 mainly nitrogenous were reduced in growth 8 percent, and the mainly potassic cultures were reduced 6 percent.

DISCUSSION

The data presented in connection with the foregoing experiments are interesting in that they again show that the physiological effect of organic compounds on plant development and growth are altered by inorganic salts. The study of crotonic acid adds knowledge of another organic compound to the list of those which have been studied in this laboratory.[5] Its behavior in regard to the action of phosphates is somewhat like that of cumarin,[6] and of certain of the aldehydes.[7] Cumarin is harmful to plants, having a characteristic effect, producing stunted tops and distorted leaves. Like crotonic acid these effects are overcome or greatly lessened by phosphates. The harmful effect of salicylic and certain of the other aldehydes is also influenced by phosphates. In soil this compound is oxidized or otherwise destroyed by phosphate fertilization. It is also interesting to note that the harmful effects of other compounds are influenced by nitrogen, and those of still others by potash.[6]

[5] Schreiner O., and Skinner J. J. Nitrogenous soil constituents and their bearing on soil fertility. U. S. Dept. Agr. Bur. Soils, Bull. 87. 1912.

[6] Schreiner, O., and Skinner, J. J. The toxic action of organic compounds as modified by fertilizer salts. Bot. Gaz. **54**: 31. 1912. Skinner J. J. Influence of phosphates on the toxic action of cumarin. Bot. Gaz. **54**: 245. 1912.

[7] Schreiner, O., and Skinner, J. J. Harmful effects of aldehydes in soils. U. S. Dept. Agr. Bull. 108. 1914. Skinner, J. J. Field test with a toxic soil constituent: Vanillin. U. S. Dept. Agr. Bull. 164. 1915. Skinner, J. J. Soil aldehydes. A scientific study of a new class of soil constituents unfavorable to crops, their occurrence, properties and elimination in practical agriculture. Journ. Franklin Inst. **186**: 165. 1918.

In regard to the exact mechanism of the chemical or physiological character of the interactions between crotonic acid and the nutrient salts, nothing definite can be said. The work thus far shows unquestionably that phosphates have an effect on the behavior of the crotonic acid towards plants; the data as a whole point to the conclusion that the antitoxic action of phosphate salts on crotonic acid is due mainly to the phosphate radical, either without or within the plant. It is also evident that the crotonic acid is less harmful in solutions of an alkaline character. The four experiments in which the crotonic acid was used in concentration of 25 parts per million were not all conducted at the same time. Each set of solutions containing crotonic acid is comparable only with the normal set which was run simultaneously. In the set of solutions in which calcium acid phosphate was used, the 66 cultures containing the crotonic acid produced 35 percent less growth than the 66 corresponding cultures without the organic acid. A reduction of 28 percent in growth is noted between the 66 normal and the crotonic acid cultures where the mono-sodium phosphate was used, 18 percent between the two sets of 66 cultures where the neutral di-sodium phosphate was used, and a reduction of only 7 percent in the two sets of 66 cultures where the alkaline tri-sodium phosphate was used. In each case, however, the crotonic acid was more harmful in the solutions where the phosphate was absent or low; the toxicity decreased as the phosphate content increased. This was the case whether the phosphate was in the form of the calcium or sodium salt, or whether it was an acid, neutral, or alkaline salt.

The metabolism of the plant as affected by the crotonic acid was studied by analyzing the solutions so as to determine the amount of P_2O_5, NO_3, and K_2O absorbed. In each of the experiments the plants in the crotonic acid solutions absorbed less P_2O_5, NO_3 and K_2O than did those in the corresponding culture containing no crotonic acid. The decreased absorption of each constituent was approximately the same. There seemed to be no interference with the absorption of any particular constituent more than with that of another.

In the earlier work with cumarin, salicylic aldehyde, vanillin, and quinone, the absorption of phosphate from solutions containing cumarin or salicylic aldehyde was more normal than that of nitrates or potash; the absorption of nitrates from cultures containing vanillin or dihydroxystearic acid was more normal than was the absorption of phosphates or potassium; and the absorption of potassium from cultures containing quinone was more normal than was the absorption of nitrates or phosphates. It is apparent that such compounds show markedly different physiological properties and are very differently influenced by inorganic salts. Whether this physiological antagonistic action of phosphates towards crotonic acid is a direct action of the salt on the organic compound or whether it acts through the medium of the plant cell cannot be definitely stated.

Summary

Alpha-crotonic acid in amounts of 25 and 50 parts per million was found to be very harmful to wheat plants grown in nutrient culture solutions. The solutions were composed of calcium acid phosphate, sodium nitrate, and potassium sulphate, and were prepared according to the triangular system. Growth was reduced about 50 percent when the crotonic acid was used in amounts of 50 parts per million and 35 percent in concentration of 25 parts per million.

Phosphates had an ameliorating effect on the harmfulness of crotonic acid. Where large amounts of P_2O_5 were present in the nutrient solution, the effect of the crotonic acid was milder than in those solutions which contained a smaller amount of P_2O_5. The toxic action of the organic compound in nutrient solutions decreased as the content of P_2O_5 increased. Experiments using NaH_2PO_4, Na_2HPO_4, and Na_3PO_4 in the place of $CaH_4(PO_4)_2$, showed that each of these phosphate salts, regardless of the basic or acidic character of the salt, had an action antagonistic to the harmfulness of alpha-crotonic acid, which seems to show that the antagonistic action of the phosphate salts toward crotonic acid is due to the phosphate radical. It is also shown that the effect of crotonic acid is less severe in solutions containing alkaline salts.

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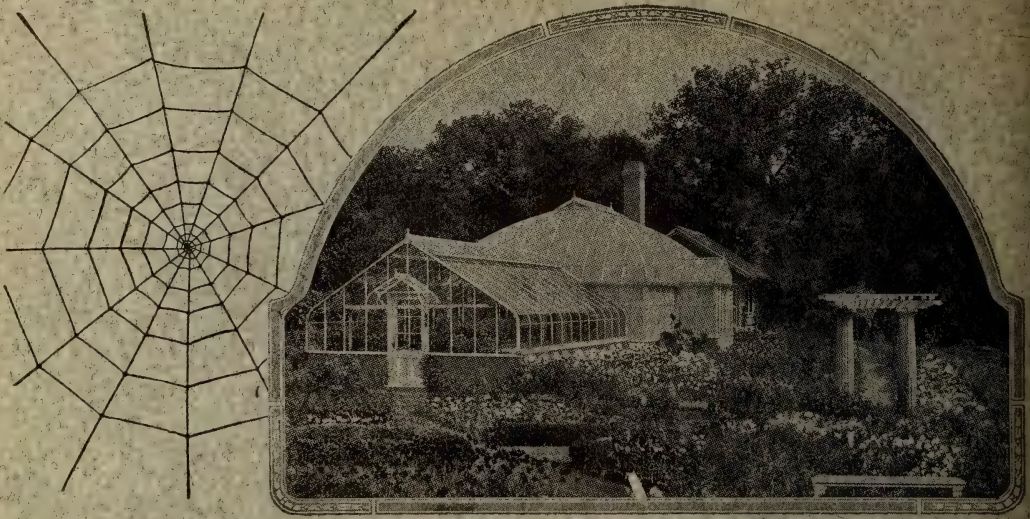

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AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

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PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT 41 NORTH QUEEN STREET, LANCASTER, PA.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania,
under the act of March 3, 1879

AMERICAN JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

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AMERICAN
JOURNAL OF BOTANY

VOL. VI MAY, 1919 No. 5

OSMOTIC PRESSURES IN THE POTATO PLANT AT VARIOUS STAGES OF GROWTH

B. F. LUTMAN

The study of concentration of cell sap in plants has followed, in general, two paths: (1) the relation of the osmotic strength to the physical environment of the plant, and (2) the part which the varying pressures in the roots, stems, and leaves play in the rise of sap.

Since the publication of the paper by Drabble and Drabble (7) on the relation of the osmotic strength of cell sap in plants to their surrounding conditions, other authors have studied the same problem, especially under the extremes to which desert vegetation is subject, since it is in desert plants that the pressure is at a maximum.

The other phase of this study has dealt with the relation of osmotic pressure to sap flow. Dixon (4, 5, 6), either alone or in collaboration with other investigators, has made the most extensive contributions to the solution of this side of the problem, although the work of Hanning (9) should also be mentioned. Dixon and Atkins' (5) observations are so closely related to those made in this paper that they require more than a passing mention.

The osmotic pressure should be higher in the leaves at the top of a tree than in those at its base, if Dixon's theory of the upward pull in the columns of sap in the water-conducting tissue is correct. His observations in general confirm this hypothesis, although there are occasional discrepancies due, as he believes, to the resistance in the conducting tracts. One of his most consistent series of observations, secured with *Wistaria sinensis* leaves, furnishes the following data:

Shaded leaves from 3 feet level,	0.412°	depression,	4.95	atmospheres
Exposed leaves from 3 feet level,	0.437°	"	5.25	"
Exposed leaves from 27 feet level,	0.550°	"	6.61	"

Similar records secured with *Ulmus campestris* show:

Leaves from short shoots at 18 feet level,	0.888°	depression,	10.68	atmospheres
Leaves from short shoots at 1 foot level,	0.763	"	9.18	"
Leaves from short shoots at outer end of arched branch in shade at 10 feet level,	1.030°	"	12.39	"
Leaves from short shoots on trunk in sunny position at 10 feet level,	1.550°	"	18.64	"

[The **Journal** for April (**6**: 131–180) was issued May 1, 1919.]

The first two observations on leaves of Ulmus are comparable since the leaves were situated under similar conditions; the last two records were obtained from leaves from the outside of the crown of the tree in one case and from a very sunny position in the last instance. Shade seems to lower the osmotic pressure even more than a location at the extreme tip of the tree increases it.

The effect of shade on the osmotic strength of the cell sap was even more clearly brought out by a series of observations on the leaves of *Syringa vulgaris:*

Covered 6 days,	1.263°	depression,	15.20	atmospheres
Exposed 6 days,	1.470°	"	17.68	"
Covered 12 days,	1.010°	"	12.15	"
Exposed 12 days,	1.608°	"	19.34	"
Covered 21 days,	.963°	"	11.58	"
Exposed 21 days,	1.505°	"	18.10	"

Dixon says, regarding other observations on the sap from the roots as compared to that of the leaves: "In Syringa the pressure of the sap of exposed leaves was found to vary from 14 to 24 atmospheres, while that of the roots lay between 4 and 6 atmospheres. In Eucalyptus the osmotic pressure of the leaves ranged between 6.1 and 8.4 atmospheres; that of the roots was 5.3 atmospheres."

The effect of wilting on the leaves of *Syringa vulgaris* is as follows:

Control,	1.352°	depression,	16.26	atmospheres
Exposed to light without water supply for 4 hours,	2.002°	"	24.07	"
Exposed to light with water supply for four hours,	1.586°	"	19.08	"

Dixon states as one of his conclusions that: "Other things being equal, mature leaves showed a higher osmotic pressure than developing leaves." No detailed study could have been made at this time as the observations were confined to the months of September and October and the latter part of August.

Dixon and Atkins (6), at a later time, observed the osmotic pressure in the leaves of *Syringa vulgaris* throughout an entire growing season and found that the rise was constant after April but that a rapid decline occurred a few weeks before the leaves dropped from the stem.

In his book on "Transpiration and ascent of sap" Dixon (4) in a footnote says: "In almost every case it was found that the older leaves, *caeteris paribus*, had a higher osmotic pressure on the same plant. This was observed in *Syringa vulgaris*, *Vitis Veitchi*, *Eucalyptus globulus*, *Hedera helix*, and especially in *Ilex aquifolium*. The leaves of the last named evergreen persist through four or five periods of growth, and it is generally found at any time that the osmotic pressure of the sap of the leaves of each successive growth is lower than that of those which precede it."

Hanning's observations (9) on the differences between the osmotic pressure of the root sap and that from the leaves should be noted. His results were obtained by the plasmolytic method and are of value as a check on those of Dixon, who made only cryoscopic determinations. His trials were made with a long series of plants from all sorts of habitats. The pressure of the root sap in nearly all cases was found to be materially less than that of the leaves. In only 14 percent of the cases did the pressure of the sap from the roots even approximate that from the leaves, while in 51 percent the pressure of the leaf sap, as compared to that of the roots, was in the proportion of 1 : 1.25; in 12 per cent it was in the proportion of 1 : 1.5; and in 23 percent it was even greater, up to 1 : 2.

The potato plant seems thus far to have escaped attention. The only accounts of its osmotic pressures are furnished by Atkins (1), who found the depression in different tubers to range from .538 to .612 degrees, corresponding to 6.47 to 7.36 atmospheres, and by Brannon (2), who found that the sap from tubers kept in an ice box from October 31 to January 23 showed a pressure of 14.51 atmospheres while the sap from tubers kept at room temperature from October 31 to December 5 measured 7.4 atmospheres.

No detailed account seems to have been given, as yet, of the osmotic relations of any herbaceous plant throughout an entire growing season. The potato seems particularly adapted to such investigation because of its succulent leaves and stems. Furthermore, the evolution from tuber to sprout and then to foliage must involve osmotic changes, and it seemed entirely possible that the deposition of starch in the tubers and the tip-burn on the foliage may be related to pressure variations in the leaf or stalk cells.

The yearly recurrence of the physiological disease known as tip-burn was the immediate stimulus to the undertaking of this study. Even during ordinary seasons, between a third and a half of the foliage in the Vermont fields is destroyed during the latter part of July and the month of August by the intense sunlight and heat. The older plants are then affected, the younger ones escaping until they attain a certain stage of maturity, when they, too, succumb.

The formation of tubers and flowering seem to mark a sharp crisis in the life of the plant. Attention was directed by this point by Jones (11) in 1903, who said that: "Reproduction by seeds is a sexual process, that by tubers is vegetative. Both are exhaustive of vital forces. The two are, therefore, in a physiological sense opposed and cannot well be carried on at the same time. Under the natural condition of the wild plant the seed precedes; with our shorter season and intensive culture we have crowded the two processes together until they tend to overlap. That is, we have forced the tuber production back into the period which in the wild plant is given to the production of flowers and seeds. As a result, we have, just after the potato plant comes into blossom, a strained and unnatural condition; a state of physiological tension, of stress between two opposing vital tendencies. According to the mode of its ancestors the major part of the plant's energy would then be tending upward toward flower and seed; but tuber production in the high-bred specialized plant begins immediately, and the acquired tendency is for this process to claim the major part of the food.

"As a result of this conflict of tendencies in the plant there occurs a *critical period* during which the continued health of the plant, if not its very life, hangs in the balance.

"Whether this explanation is correct or not, the fact is certain that the fortnight including and immediately following the blossoming period is the turning point, the crisis in the life of the potato plant."

The soil of the region around Burlington, Vermont, is in the main a light sandy loam. On this soil tip-burn, which is the external evidence of the crisis through which the plant passes, is severe. No better location, therefore, could be found for making observations on the internal factors, chemical and physical, that are at play inside the potato plant during the growing season. However, the crisis mentioned by Jones is more than a fortnight long; it lasts at least a month or six weeks, as the observations made during the course of the present work will show.

The osmotic pressures in the plant are the result of the presence of sugars or inorganic salts in solution in the cell sap. So far as could be ascertained no analysis of the sap itself was available, but the ash of the plant has been investigated a number of times.

Choslowski (3) found considerable percentages of glucoses early in the season both in the pith and in the vascular portions of the potato stalk. After June 28 only the pith contained glucoses. After August 26, two weeks before the death of the plants, the percentage of sugars in the stems had diminished. The young tubers and their stolons were very rich in glucose early in the season, but after June 28 showed little sugar. He attempts no explanation as to their movement other than to state that they move in a diosmotic manner.

Kellermann (12) determined that the calcium, potassium, and phosphorus in the ash of potato leaves and stalks increased to a certain maximum during the summer and then rapidly fell away during the last few weeks of growth.

Seissl and Gross (15, 16) found that the potato leaf ash contained a maximum of P_2O_5 and K_2O on July 1 and that the percentages decreased from that date until the harvest, October 10. The same investigators also found generally a maximum percentage of CaO, MgO, K_2O, SO_3, and P_2O_5 in the leaf ash on either July 1 or August 1, with a decrease after the latter date. A few exceptions to this rule can be found in their tables of analyses, but these are due to specific fertilizer applications. The late summer and autumnal decreases in CaO, MgO, SO_3, and P_2O_5 percentages were especially marked with the Johannis, while the decrease in P_2O_5 content was notable in both Johannis and Perkun, whatever the fertilization.

METHODS

The plants were brought in from the field or garden during the summer of 1918, and the sap was immediately extracted from the leaves, stems, or tubers by grinding up these organs in a small Excelsior food chopper and pressing the juice through cheesecloth. The requisite 12–15 c.c. needed were usually easily obtained as all the organs except the roots are very succulent. The sediment was allowed to settle and the upper portion decanted off. The freezing was done with the help of an ordinary Beckmann thermometer and apparatus. At least two determinations were made in each case, and if no super-cooling appeared, the trials were repeated three or more times. It was impossible to obtain any super-cooling with a few samples of leaf juice, but enough attempts were made so that the true freezing point was closely approximated. Correction of the depressions was made for super-cooling using the formula suggested by Harris and Gortner (10), and their tables have been employed in converting the depressions in degrees into atmospheres. In a number of cases, the juice was analyzed for glucoses (or reducing sugars) and sucrose by the gravimetric Fehling's method. The presence of various organic materials in the juices introduced disturbing factors, but the determinations at least afford some idea of sugar percentages. Complete analyses were made of four juices. The author acknowledges the help of Mr. R. L. Gale, who had general charge of the analytical work and who assisted with the cryoscopic readings.

Green Mountain potatoes were used in all cases unless otherwise stated.

COMMENTS ON OBSERVATIONS

The freezing point of the tuber sap varied considerably, as might be expected in view of the conditions under which the tubers were kept. However, in every case the pressure exceeded 7 atmospheres. These depressions were greater than those obtained by Atkins (1) but correspond very closely to those secured by Brannon (2) for other varieties of potatoes kept at room temperatures. The pressure in the sprouts was even more variable, covering a range of from 6 to 12 atmospheres. The sprouts used in reading no. 3 (table 1) were obtained from the tubers used in reading no. 2. It will be seen that the pressure in the sprouts was 8.75 atmospheres while that in the tubers was 7.691 atmospheres. The sugars were more than twice as abundant in the sprouts as in the tubers and were the cause of the increased osmotic pressure.

The osmotic pressure of the sap of the very young plants was not determined during June and early July. The first records were secured on July 18 when the plants were full grown, in bloom, and with tubers a centimeter or a centimeter and a half in diameter. No tip-burn had appeared as yet, and the plants were turgid and healthy. The results secured on this particular plant indicate the greatest pressure in the stalks. The weather

TABLE I

No.	Date (1918)	Weather	Material	Depression	Depression in Atm.	Percent Glucose	Percent Sucrose	Remarks
1	June 9		Green Mt. tubers kept in dry room.	.856	10.30	.497	.769	
2	July 5		Green Mt. tubers from greenhouse cellar.	.639	7.69	.946	.950	
3	" 5		Sprouts from above.	.727	8.75	2.32	2.25	
4	June 14		Sprouts from tubers in barrel.	.985	11.86			
5	" 17		Sprouts from tubers in boxes.	.876	10.55			
6	" 17		Long, slender sprouts.	.510	6.14			
7	8 A.M. July 18	A bit cloudy. Rain on July 17.	New leaves.	.434	5.23	6.16	Undet.	No tip-burn.
8	"		Old leaves.	.523	6.30			
9	"		Stalks.	.626	7.54			
10	"		Tubers.	.453	5.46	.508	.899	
11	4 P.M. July 20	Clear. Two preceding days hot and clear.	New leaves.	.670	8.07	.484	.881	No tip-burn.
12	"		Old leaves.	.653	7.84	.185	.208	
13	"		Stalks.	.670	8.07	.228	1.115	
14	"		Tubers.	.506	6.10	.299	.873	
15	8 A.M. July 22	Hot, cloudy. Warm and clear on preceding day.	New leaves.	.567	6.83	.000	trace	Tip-burn begins.
16	"		Old leaves.	.604	7.28	.207	.067	
17	"		Stalks.	.622	7.49	.171	.278	
18	"		Tubers.	.492	5.93	.000	.306	
19	July 23	Slightly hazy, but hot with high humidity.	New leaves.	.691	8.32	1.333	.090	Tip-burn shows from preceding day.
20	"		Old leaves.	.631	7.60	.909	.324	
21	"		Stalks.	.720	8.67	1.429	.000	
22	"		Tubers.	.527	6.35	.444	1.161	
23	5 P.M. July 25	Previous day warm and with sunshine.	New leaves.	.536	6.46	1.020	.000	Continued tip-burn.
24	"		Old leaves.	.568	6.84	1.068	.000	

TABLE 1 (*Continued*)

No.	Date (1918)	Weather	Material	Depression	Depression in Atm.	Percent Glucose	Percent Sucrose	Remarks
25	5 P.M. July 25		Stalks.	.703	8.47	1.016	.021	
26	"		Tubers.	.412	4.96	.588	.706	
27	10.45 P.M. July 25	After a bright, hot day.	New leaves.	.640	7.71	.1714	Undet.	
28	"		Old leaves.	.602	7.25	.000	.000	
29	"		Stalks.	.781	9.41	.186	2.146	
30	"		Tubers.	.541	6.52	.000	1.073	
31	9.30 A.M. July 27	Hazy, but hot. Preceding day fair.	1st row of greenhouse plot. New leaves.	.754	9.08			
32	"		Old leaves.	.732	8.82			
33	"		Stalks.	.828	9.97			
34	"		Tubers.	.594	7.16			
35	"		2nd row. New leaves.	.754	9.08			
36	"		Old leaves.	.689	8.30			
37	"		Stalks.	.944	11.37			
38	"		Tubers.	.587	7.07			
39	11 A.M. July 29	Hazy but hot.	New leaves.	.742	8.95	.000	.201	Plants a little wilted from the intense heat of the previous day. Tip-burn advances.
40	"	Previous day hot and dry.	Old leaves.	.710	8.55	.000	.235	
41	"		Stalks.	.886	10.70	.000	.766	
42	"		Tubers.	.533	6.42	.000	.489	
43	10 A.M. July 30	Clear. Heavy rain previous night.	New leaves.	.539	6.49	.009	.755	Plants turgid.
44	"		Old leaves.	.488	5.88	.000	.544	
45	"		Stalks.	.821	9.89	.072	2.270	
46	"		Tubers.	.486	5.86	.000	.000	
47	5 A.M. July 31	Cool, partly cloudy. Cloudy preceding day.	New leaves.	.514	6.19	.019	.023	
48	"		Old leaves.	.522	6.29	.000	.000	

TABLE 1 (*Continued*)

No.	Date (1918)	Weather	Material	Depression	Depression in Atm.	Percent Glucose	Percent Sucrose	Remarks
49	5 A.M. July 31		Stalks.	.874	10.53	.000	.027	
50	"		Tubers.	.489	5.89	.000	Trace	
51	9 A.M. July 31		Corn leaves.	.569	6.85	.028	.032	
52	"		Corn stalks.	.624	7.52	.017	Trace	
53	"		Bean leaves.	.792	9.54	.009	.000	
54	"		Bean stalks.	.918	10.94	.000	.064	
55	"		Bean pods and beans.	.716	8.62	.045	.000	
56	Aug. 7	Warm and fairly bright.	Leaves of young plant.	.606	7.30	.000	Trace	Flower buds showing and tubers beginning to form.
57	"		Stalks, young plant.	.579	6.98	.000	"	
58	"		Seed piece, plant.	.566	6.82	.000	.752	
59	"		Leaves, old plant.	.749	9.02	.000	.000	With large tubers and flowers almost past.
60	"		Stalks, old plant.	.840	10.12	.006	2.064	
61	2.30 P.M. Aug. 8	Cloudy, warm, and humid. Rain previous night.	Leaves, young plant.	.672	8.08			
62	"		Stalks, " "	.684	8.24			
63	"		Leaves, old plant.	.754	9.08			
64	"		Stalks, " "	.824	9.92			
65	"		Tubers, " "	.554	6.67			
66	2.30 P.M. Aug. 17	Cold, brilliant sunshine.	Inside of leaflet of young plant.	.868	10.45	.000	.076	
67	"		Periphery of leaflet of young plant.	.925	11.14	.000	1.447	
68	"		Stalks, young plant.	.685	8.25	.000	.000	
69	"		Inside of leaflet, old plant.	.787	.948	.000	.546	
70	"		Periphery of leaflet of old plant.	.836	10.08	.000	1.236	
71	"		Stalks, old plant.	.821	9.89	.000	.000	
72	11 A.M. Aug. 19	Cold, brilliant sunshine.	Sunflower leaves, inside.	1.026	12.35	.383	.939	
73	"		Sunflower leaves, outside.	.940	11.32	.393	.450	
74	4.30 P.M. Aug. 19	" " "	Inside of leaves, old plants.	.877	10.56	.000	.460	

TABLE 1 (*Continued*)

No.	Date (1918)	Weather	Material	Depression	Depression in Atm.	Percent Glucose	Percent Sucrose	Remarks
75	4.30 P.M. Aug. 19		Periphery of leaves, old plants.	.992	11.93	.000	1.054	
76	8.30 A.M. Aug. 20	Cool, brilliant sunshine.	Tips of leaflets, old plants.	.957	11.52	.000	.479	
77	"		Butts, old plants.	.848	10.22	.000	.000	
78	2 P.M. Aug. 22	Clear and warm.	Inside of leaflets, old plants.	.845	10.19			
79	"		Periphery of leaves, old plants.	.992	11.94			
80	9 A.M. Aug. 27	Clear and warm.	Dahlia leaves.	.557	6.71			
81	"		" stalks.	.541	6.52			
82	"		" tubers.	.549	6.61			
83	2 P.M. Aug. 27	Clear and bright.	Young leaves.	.692	8.34			
84	"		Old "	.717	8.63			
85	"		Wilted "	.837	10.08			
86	9.30 A.M. Aug. 28	Clear and bright.	Chicory leaves.	.870	10.48			
87	"		" stalks.	.840	10.12			
88	"		" roots.	.712	8.58			
89	2 P.M. Sept. 1	Cool with brilliant sunshine.	Leaves, old plant	.810	9.75	.000	.067	Plants were about a third dead from tip-burn. Some new growth. For complete analysis, see Table 2.
90	"		Stalks, " "	.746	8.98	.000	.000	
91	"		Tubers, " "	.511	6.16	.000	.000	
92	"		Leaves, young plant.	.580	6.99	.000	.000	
93	"		Stalks, " "	.561	6.76	.000	.000	
94	2 P.M. Sept. 9	Cool with brilliant sunshine.	Leaves, young mosaic plants.	.650	7.83	.660	.000	
95	"		Leaves of plants shaded 48 hours.	.522	6.29	.000	.000	
96	"		Stalks of plants shaded 48 hours.	.538	6.48	.000	.000	

TABLE 1 (*Continued*)

No.	Date (1918)	Weather	Material	Depression	Depression in Atm.	Percent Glucose	Percent Sucrose	Remarks
97	Sept. 18	Cool and cloudy. Cloudy preceding day.	Old (green) stalks	.502	6.05	.000	.000	Still with some green leaves. For complete analysis, see Table 2.
98	"		Old (yellow) stalks.	.354	4.27	.000	.000	
99	11 A.M. Sept. 19	Clear and cool.	Young leaves of old McCormick plants.	.633	7.63			After continued rains.
100	"		Part of upper stalk	.671	8.08			
101	"		" lower "	.660	7.95			
102	"		Roots.	.355	4.28			
103	"		Tubers.	.557	6.71			
104	"		Balls.	.534	6.44			Stalks of fruit dead in some cases.
105	"		Young leaves (wilted).	.885	10.66			
106	"		Upper stem (wilted).	.786	9.46			
107	Sept. 24	Cold. Rain for two weeks.	Leaves, old plant (Green Mt.).	.645	7.77			After long rains with little sunshine.
108	"		Stalks, " "	.616	7.42			
109	"		Tubers, " " (Green Mt.).	.528	6.36			
110	"		Leaves, young plants.	.686	8.26	1.386	.359	Growing since about August 7.
111	"		Stalks, " "	.680	8.19	1.466	.613	
112	"		Seed piece.	.532	6.41	2.610	.172	
113	"		Tubers, young plants.	.589	7.09	.816	1.796	
114	Sept. 24	Rain for two weeks.	Tomato leaves (Yellow Plum).	.633	7.64			
115	"		" stalks.	.647	7.79			
116	"		" green fruit.	.557	6.70			
117	"		" ripe "	.620	7.46			
118	10 A.M. Sept. 28	Cool and cloudy.	Garden beet leaves.	.733	8.83	.000	.000	
119	"		Garden beets.	.981	11.81	.000	5.590	
120	"		Carrot leaves.	.980	11.80			
121	"		Carrots (McCormick).	.812	9.77			
122	"		New leaves.	.658	7.93			Old plants, growing all summer, but recently putting out new foliage.

TABLE I (*Continued*)

No.	Date (1918)	Weather	Material	Depression	Depression in Atm.	Percent Glucose	Percent Sucrose	Remarks
123	Sept. 28		Stems.	.598	7.20			
124	"		Tubers.	.566	6.80			
125	5 P.M. Oct. 1	Clear and cool. Rain previous night.	Leaves (normal).	.780	9.39	.202	.384	Plants set out about Aug. 7. Growth had ceased and tubers were being formed.
126	"		Stalks "	.689	8.30	.535	.048	
127	"		Leaves (mosaic).	.831	10.00	.192	1.186	Plants similar to above; leaves dying in spots.
128	"		Stalks "	.631	7.60	.277	.331	
129	"		Sugar beet leaves (outer).	.801	9.65			Outer leaves turning yellow.
130	"		Sugar beet leaves (inner).	.949	11.42			
131	"		Sugar beets.	1.429	17.18	.000	12.626	
132	"		Sugar beet leaves.	1.014	12.21	1.280	.000	
133	"		Ripe tomatoes (Yellow Plum).	.617	7.43			Very ripe fruit.

of the two preceding days had been rainy. Later observations revealed the fact that such atmospheric conditions tend to lower the pressure in the leaves while that in the tubers and stems remains almost constant. The records made two days later are, therefore, to be taken as representing more nearly the relative pressures in the various parts of the plant during average July weather. At that time the pressures had all risen, but particularly that of the leaves. Two hot, clear days had intervened. In the latter case, the stalks displayed a high osmotic pressure but it was not much above that of the leaves. The same observations can be made on the determinations of July 22 when tip-burn began to be noticed on some of the plants.

A period of intense heat and extensive tip-burn injury then ensued. The sap in the leaves and stalks exhibited a much smaller depression when obtained at 5 A.M. on July 25 than that at 5 P. M. on July 23, 10:45 P.M. July 25, 9:30 A.M. July 27, or 11 A.M. July 29. The great preponderance of the pressure in the juice from the stalks is the striking feature of the observations made during this intensely hot week; in one instance this pressure increased to 11.37 atmospheres. The tuber sap pressure seemed to be more stable, although in one case it rose to 7.16 atmospheres. An analysis of the juices revealed a high sugar content in the stalks at all times, 2.146 percent of sucrose on July 25 being the maximum. The osmotic pressure in the tubers must also be largely maintained by sugars, both reducing sugars and sucrose being always present in large amounts.

A heavy rain on the night of July 29 broke the hot spell of weather, and the response of the plants was almost immediate, as the observations made at 10 A.M. on July 30 indicate. The sap in the stalks, with a sucrose content of 2.27 percent, did not change as rapidly, although the pressure was somewhat less than it had been the previous day.

The high pressure in the juice from the stalks was at 5 A.M. July 31, but the pressures in the juices of the leaves and tubers were approximately the same as those obtained at 10 A.M. the preceding day.

The next observation was made on August 7, when the sap from young plants was compared with that from fully grown specimens. The striking fact here presents itself that in the young plants all the organs exhibited nearly the same osmotic pressure and that the leaves take the lead by about a half-atmosphere. The juice from the old seed tuber, as might be expected, produced the smallest depression. The pressure of sap from the stalks of the old plants was almost an atmosphere greater than that from the leaves. The relatively high sucrose percentage in the stalks should be noted.

A repetition of this series of observations was made at 2:30 P.M. August 8. The stalk sap, in this case, recorded a slightly larger depression than the leaf sap, but the difference between the juices of the old leaves and those of the stems amounted to over 0.8 atmosphere.

The trials made on August 17 were meant to determine the osmotic pressure in the sap of the periphery of the leaflets as compared to that near the midribs. The leaflets were cut as shown in Fig. 1. Both old and young plants were used. Errors were introduced into the determination by the evaporation from the cut surfaces, but the readings were at least comparative. The sap obtained from the periphery of the leaflets from both young and old plants recorded a higher osmotic pressure than the sap secured from the region of the midrib. The high percentage of sucrose in the peripheral portions must also be noted. Similar trials were made on August 19, when even greater preponderances in peripheral osmotic pressures and sucrose contents were evidenced. Similar cryoscopic results were recorded on August 22, although the sugar determinations were not made.

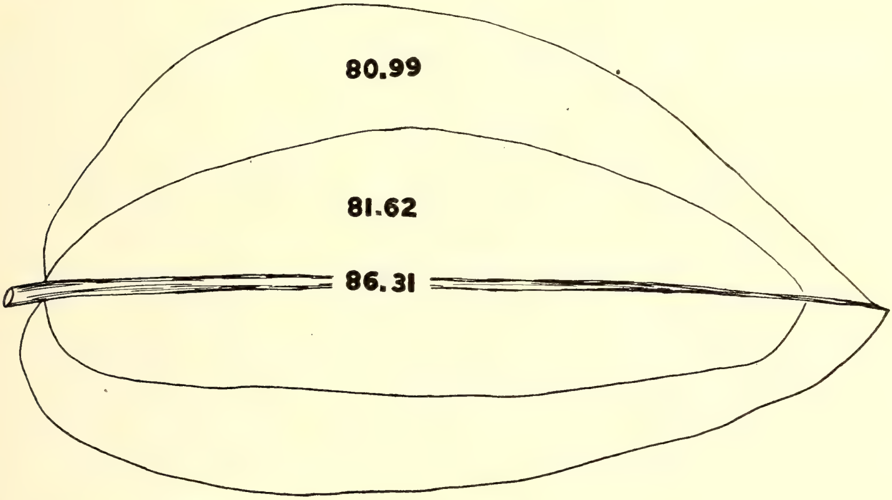

FIG. 1. Potato leaflet, percentage of water in the periphery, near the midrib, and in the midrib.

Sunflower leaves were similarly tested on August 19, and showed reverse results both as to pressure and sucrose percentages.

A determination was made on August 20 of the depressions of the saps secured from the tips and from the butts of the leaflets. The tips showed over an atmosphere greater pressure than the butts, due to higher sucrose percentages.

The water content of the leaflets was determined by cutting them up into small pieces as indicated in Fig. 2, weighing, drying at 100° C. for 4 to 5 hours and reweighing. While the leaflet periphery contained sap of a higher osmotic pressure than that in the midrib (2), the leaflet really is much more succulent along the midrib and toward the base than it is toward its outside. The leaflet, in spite of the higher peripheral osmotic pressure, loses water from these parts at such a high rate on hot days that these portions are often found to be wilted.

A large dahlia plant with almost full grown flower buds, used on August 27, seemed to be at an osmotic equilibrium.

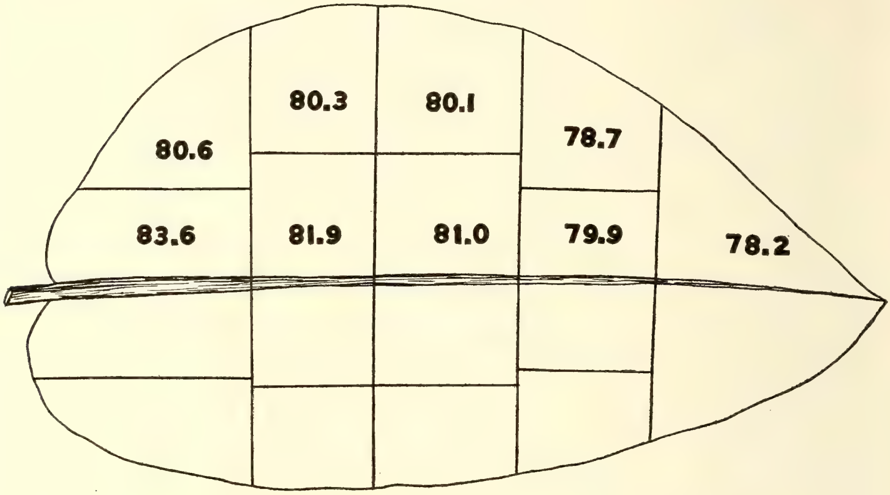

FIG. 2. Potato leaflet, percentage of water in various portions of the lamina.

Chicory plants (August 28) showed an osmotic pressure of almost two atmospheres more in the leaf than in the root sap, with the stalk sap intermediate between the two. Chicory behaved in every way as would be expected if, as a matter of fact, osmotic pressure is the cause of the upward sap flow.

Heavy rains occurred during early September, cool weather followed, and the older potato plants that had not entirely succumbed to tip-burn began to resume growth. Cryoscopic readings were taken on September 9 of the juice from an old plant and from some young plants growing in a parallel row since the first week in August. The striking features at this time were the relative pressures in the leaves and stalks, the pressure in the tubers having remained about constant. The osmotic pressure in the leaf sap had greatly increased, so much so, in fact, that it exceeded that of the stems by 0.77 atmosphere. The young plants corroborated the readings of August 7 and 8, the leaf sap pressure being greater than that of the stalks. The increase in pressure in the old plant seems largely due to the inorganic salts soluble in the cell sap, since the sugars were conspicuously absent at this time. The cooler weather and lack of sunshine were probably responsible for the latter condition. A complete analysis was made of the ash of the sap used in these cryoscopic determinations (table 2).

TABLE 2.

PERCENTAGE OF WATER SOLUBLE SALTS IN JUICES.

Sept. 9, 1918. Old plants.

	P_2O_5	Cl	SO_3	CaO	MgO	Na_2O	K_2O	Total
Leaves	.496	.180	.128	.353	2.260	.133	.120	3.671
Stalks	.558	.192	.139	.378	1.487	.035	.359	3.148
Tubers	.821	.046	.241	.394	1.111	.096	.071	2.761

Sept. 9, 1918. Young plants.

	P_2O_5	Cl	SO_3	CaO	MgO	Na_2O	K_2O	Total
Leaves	.472	.061	.235	.186	.871	.044	.065	1.935
Stalks	.205	.053	.040	.256	1.013	.073	.075	1.715

The foliage on many of the old plants was largely dead by September 18, but the stalks, together with small tufts of leaves, were still green. Other plants had lost their foliage entirely and their stalks were of a yellow-green hue. The diminution in the osmotic pressure of the sap from these stalks that were still green is marked, but it is even more so in the yellow-green ones. The sugars were entirely absent. Analyses of the ash of the saps appear in table 3.

TABLE 3.

PERCENTAGE OF SALTS IN JUICE OF STALKS

Sept. 18, 1918.

	Old Stalks	Green Stalks
CaO =	.949%	2.212%
SO_3 =	.029%	.207%
MgO =	1.364%	1.815%
Cl	.076%	.142%
P_2O_5 =	.552%	.603%
Na_2O =	.089%	.142%
K_2O =	.088%	.130%
	3.141% ash.	5.251% ash.

It will be seen that as the foliage and stalks die and the tubers mature, the latter withdraw from the aerial portions a considerable portion of the soluble materials. These results on the ash of the soluble salts in the sap agree with those secured by Kellerman (12), noted before.

The McCormick plants used on September 19 still showed a slightly higher osmotic pressure in the stalk sap than in the leaf sap, the sap in the upper part of the stalk did not differ materially from that in the lower part, the roots exhibited a remarkably low pressure, while the fruit and tubers occupied in this respect an intermediate position between the leaves and roots. Two of the plants were allowed to transpire in a current of air for five hours, and the freezing points of the juice from the leaves and the upper part of the stalk were again determined. The incipient wilting had raised the osmotic pressure of the leaf sap 3 atmospheres or 39 percent, while in the stalks the increase was only 1.38 atmospheres or 17 percent. On August 27 a similar experiment had been made on leaves removed from the plant and allowed to transpire for 2½ hours. The increase in this instance was 1.6 atmospheres or about 18 percent.

The young Green Mountain plants, growing since about August 7, had developed tubers a centimeter or more in diameter in about seven weeks. The leaves and stalks exhibited about the same pressure, while that of the tubers was rather higher than usual and that of the seed piece was very low. The large amount of sugar present in all the organs was conspicuous and is comparable to that in the plants, used on July 20, 22, and 23, which were in about the same stage of development. The increase in the osmotic pressure in all parts of the plant, too, shows that the soluble materials at this stage of growth had about reached their maximum.

The only other observations on normal potatoes were made on September 28, on some old McCormick plants that had started to put out new foliage. The new leaves at this date contained a sap with an osmotic pressure 0.73 atmosphere higher than the sap of the stems. On September 19, the reverse had been true.

Shading the potato plant diminishes the osmotic pressure of the sap of the leaves and stalks. The pressure in the leaves of a normal young plant on September 9 was 6.99 atmospheres, while that found in a plant shaded for 48 hours was only 6.29 atmospheres. In the stalks of the same plants, the pressures were 6.76 and 6.48 atmospheres. Shading, therefore, by lowering the osmotic pressure would enable parasitic fungi more readily to obtain their food material and to increase their rate of growth. Dark, cloudy, rainy days would be very effectual to this end, since increased moisture seems to lower the pressure, as is clearly shown by the observations on July 27 and 29 taken before a rain, and on July 30 and 31 after a rain. The relation of lowered osmotic pressure to the spread of epidemics due to fungi has not been heretofore recognized clearly, although Dixon and Atkins (5) have already pointed out that shading had this effect on the leaves of Syringa:

Covered, 2 days,	21.63 atmospheres
Exposed, 2 days,	24.57 "
Covered, 3 days,	19.03 "
Exposed, 3 days,	20.00 "
Covered, 7 days,	15.97 "
Exposed, 7 days,	19.12 "

The effect of the weather was also noted by them on the same plant:

Leaves, gathered after a dark day, no sunshine, 16.26 atmospheres
Leaves, gathered after a bright day, 9 hours' sunshine, 22.40 atmospheres
Leaves, gathered after a bright day, 7 hours' sunshine, 20.40 atmospheres

The checking of epidemics of *Phytophthora infestans* by hot, dry weather is usually ascribed to a "drying up" of the fungus in the leaf. This is probably literally true. The fungus may be able to withdraw food materials from the leaf when the pressure is only about 6 atmospheres, as on July 31, but when this pressure rises to 9 or 10 atmospheres, as on August 8, the parasite may itself lose all its water and be unable to recover.

Cryoscopic readings of the sap from plants badly affected by mosaic were made on September 9 and on October 1. The leaf sap of the mosaic plant recorded a pressure of 7.83 atmospheres, while that from a normal plant growing near it showed only 6.99 atmospheres. The mosaic leaves also contained an unusually high percentage of reducing sugars. The pressure in the normal leaves on October 1 was 9.39 atmospheres, while in the mosaic leaves it was 10 atmospheres. The much higher percentage of cane sugar should also be noted. On the other hand, the sap of the normal stalks exhibited a pressure of 8.30 atmospheres while that of the mosaic ones showed only 7.60 atmospheres. The higher pressures in the mosaic leaf sap seem to be due to the presence of abnormally large amounts of sugars. This may indicate an inability on the part of organs so affected either to transform or to transport their carbohydrates.

Comparison of the potato plant with nearly related plants, such as the tomato, or with vegetables that deposit their carbohydrates in enlarged roots, such as the carrot or the beet, ought to throw some light on the pressures in storage organs or fruits. The juices from the tomato plant tried on September 24 recorded about the same pressures in leaves and stalk. Growth at this time had ceased. The low osmotic pressure in the green fruit is peculiar but corresponds almost exactly with the pressure in the fruit from the potato on September 19.

The ripening of the tomato fruit increased its osmotic pressure 0.76 atmosphere as shown on September 24 and verified on October 1, with very ripe fruit. Unfortunately no tomato plants were used for osmotic pressure experiments during the very hot weather in August, but the probability is that the stalk sap would have shown a higher pressure than the leaf sap, as was the case with the potato in that period. Corn, on July 31, recorded a pressure of 6.85 atmospheres in the leaves and 7.52 atmospheres in the stalks, while beans gave a pressure of 9.54 atmospheres in the leaves, 10.94 atmospheres in the stalks, and only 8.62 atmospheres in the pods and beans.

Carrots, on September 28, exhibited 11.80 atmospheres pressure in the sap from the leaves and 9.77 atmospheres in the sap from the roots themselves.

Garden beets, on September 28, had 8.83 atmospheres pressure in the leaf sap and 11.81 atmospheres in the beet root sap. The 7.5 percent of cane sugar in the root explains the unusually high pressure. Sugar beets and sugar beet leaves were tried on October 1. The inner leaves were still growing and their sap had a pressure of 11.42 atmospheres while the outer ones gave only 9.65 atmospheres. Another lot of beets recorded 12.21 atmospheres for the leaves and 17.18 atmospheres for the roots. The latter pressure is developed from the 12.6 percent of cane sugar of the sap, while the 1.28 percent of reducing sugars in the leaves helped to maintain the equilibrium to some extent.

General Discussion

A *résumé* of the observations made on the osmotic pressure of the potato plant at different periods of development seems to show the following conditions to succeed each other:

1. The normal pressure in the seed tubers as they are taken from storage is between 7 and 10.3 atmospheres.
2. The sprouts which come from these tubers, not in the soil, exhibit a pressure slightly in excess of that of the tubers themselves.
3. This pressure for the tubers or seed piece is lowered by the absorption of water until it drops to 6.82 (August 7), or 6.41 atmospheres (September 24).
4. The juice of the leaves of the young plant records a higher osmotic pressure than that of the stalk, and the osmotic pressures of the juices from both leaves and stalk are greater than that of the juice from the old seed piece.
5. The osmotic pressure becomes greater in the stalk than in the leaves after the flower buds are put out and the tubers begin to grow.
6. The growing tubers maintain an almost constant pressure from the time they are of a sufficient size for the determination of pressure until maturity.
7. The pressure in the stalk is less variable than that in the leaves and continues high throughout the active tuber and starch period.
8. The return of cool, rainy weather starts growth of the foliage again, and the osmotic pressure in the leaves again becomes greater than that in the stalks.
9. The osmotic pressure in the old plants is higher than that in the young ones.
10. The pressure diminishes again in the very old plants that have lost practically all their foliage and sinks to a very low ebb in the yellow-green stalks with no foliage.

These observations have a distinct bearing on certain conclusions, both theoretical and practical.

1. A superior osmotic pressure seems to be necessary for the formation of new growth. The sprouts have a greater osmotic pressure than have the tubers from which they arise. The leaves have a higher osmotic pressure than the stalks during the early growth period while the foliage is being produced, but as soon as they lose that predominance, the growth stops and is not resumed until the predominance is again assumed, usually late in the growing season, in September.

2 A superior osmotic pressure is not necessary to maintain an organ after it has been formed. The leaves during July and August do not have as high an osmotic pressure as do the stalks, but they are able to maintain themselves and produce large quantities of starch. It seems necessary to assume, therefore, that the transpiring organs are connected directly with the root system by a long series of tubes in which the cross walls offer a very slight opposition to the hydrostatic head while their side walls are comparatively impermeable. The rapid recovery of an herbaceous plant from wilting would support this theory, as would also the sudden drop in osmotic pressure of the sap from the leaves between 11 A.M. July 29, and 10 A.M. July 30, during which period a heavy rain occurred. The plants on July 29 were in a state of incipient wilting. The water that came to them must have passed through a long zone in the stalk where the osmotic pressure was greater outside the water-conducting tubes than it was inside them; still, these leaves did not wilt to any great extent. The question of the maintenance of turgor in the leaves of plants like the beet seems to have been overlooked by Dixon (4) in his theoretical discussions of sap flow, a though the conception he presents (pp. 141–142) of transpiring leaf cells at the upper end of a long tube (the tracheae) is the correct one. The osmotic pressure in these leaf cells is a measure of the pressure in the tracheae and in the conducting tissue, but not necessarily of that in the tissues in which the water tubes are imbedded. It would seem, therefore, that to the conception of transpiring cells at the upper end of a series of tubes should be added the idea of a direct connection of these tubes with the absorbing cells of the roots. Otherwise, the beet root would pull all the water from the leaves. The pressure in the garden beet itself, according to the determination on September 28, was 11.81 atmospheres while in the leaves it was only 8.83. These pressures are comparable to those obtained by Pringsheim (14, page 135) for beets growing in damp soil, namely 11.68 atmospheres. The leaves were found by the same investigator to maintain a pressure of 15.52 atmospheres while still young, but with the growth of the leaf the pressure fell to 5.30 atmospheres, at which point it remained constant. The observations recorded in table 1 on October 1 for sugar beets and sugar beet leaves are even more divergent in their differences. The beets had a pressure of 17.18 atmospheres, while in one case the outer leaves had 9.65 and the inner, 11.42 atmospheres. All the leaves taken together from another beet plant gave 12.21 atmospheres. The soil water in the tracheae supplying the leaves from the fine rootlets must be practically impermeable to the high osmotic pressures surrounding them. Some water might be lost inward from one leaf cell to the adjacent ones nearer the stalk in the potato plant when the stalk has such a preponderance in osmotic pressure over that of the leaf, this process adding to the loss outward from transpiration. The total amount withdrawn by the cells may be small, but it may be the small excess that is necessary for the preservation

of turgor in these peripheral leaf cells. The bean and the corn plants were found to have a higher osmotic pressure in the stalks, and these plants do not often suffer from wilting of the tips and margins of the leaflets (with the resultant tip-burn) as do those of the potato plant. Several reasons may be given for this difference. The bean leaves in intense sunlight assume such a position that their laminae are parallel to the eight rays while the corn leaves roll up in intense heat. Neither of these plants is as succulent as the potato; the loss of water between adjacent cells ought to be very small as compared with that between the potato cells. The real cause for tip-burn in the potato may lie, too, in the movement of the elaborated food materials.

3. A high osmotic pressure does not seem to be necessary for the growth of reproductive organs nor for the continued deposition in them of reserve carbohydrates, such as starch or sugar. The sugar beet or the garden beet ought to be able to withdraw elaborated food materials from the leaves by osmotic pressure since their pressure is greater than that of the leaves. If these materials were put into the proper channels, they ought to find their way by osmosis to the storage organ. The continued growth of the potato tuber continues, however, when its osmotic pressure is only between 6 and 9 atmospheres, and at no time is the pressure greater in the tuber than it is in the leaves. The stalks record even a greater pressure than do the leaves. The movement of food materials is undoubtedly due to differences in osmotic pressures, but the manner in which they work to induce the flow of carbohydrates to the tubers is, as yet, unexplained.

The stalk of the plant seems to serve as an organ for temporary storage if the amount of sugar in it during periods of active carbon assimilation is an indication. Elaboration processes probably occur here but their nature is almost entirely speculation. The further translocation in the potato plant is dependent on the age of the plant; early in its growth, the material is used for the production of new leaves, but after the formation of the young tubers, the current changes its direction and the materials flow into them. The translocation of these carbohydrates probably occurs largely through the sieve-tubes according to the plant physiologists who have studied this phase of the subject; see Pfeffer (13, pp. 575–583) and Haberlandt (8, pp. 328–336). Neither of these authors, however, explains on the basis of experimental evidence the means by which the carbohydrate compounds are moved. Haberlandt (p. 334) makes the following statement concerning protein compounds: "When a petiole or stem of Cucurbita is cut across, large quantities of slimy protein-material exude from the several sieve-tubes. With reference to this point, A. Fischer has proved that the effects of a cut petiole extend through one or two internodes at the very least. This observation indicates that the pressure in the sieve-tubes is sufficient to overcome the resistance opposed by a very considerable number of sieve-plates. Thence we may infer that any differences which arise within the intact sieve-tube system, owing to the partial depletion of the tubes at certain points, are at once equalized by a more or less rapid displacement of the liquid contents in the corresponding direction. Whether the hydrostatic pressure in the sieve-tubes owes its origin to the osmotic properties of the liquid contents or whether it is due to compression of the sieve-tubes by the highly turgescent adjoining tissues (leptome-parenchyma and companion cells) is still uncertain. Most probably both factors have a share in producing the pressure observed." He concludes: "This matter evidently requires further investigation."

The movement of food materials to the growing leaves of the young plant can be explained on the basis of high osmotic pressures in these organs, but the growth of the potato berries, of the young tomatoes, and of the potato tubers cannot be accounted for in any such manner since the osmotic pressure in all of these organs is the least of any in the plant. We must fall back upon the pumping action of the sieve-tubes as suggested by Haberlandt.

One question more remains to be answered: Why does the potato plant suffer from tip-burn while the tomato is usually exempt? This may be due to structural differences in the leaves or to the resistance of the cells to incipient wilting, but it seems to be better explained by the continued movement of the elaborated carbohydrates upward in the tomato plant to form new foliage while in the potato they travel downward to form starch in the tubers. The tomato leaves may be better nourished from this food stream, and the continued formation of vigorous new leaves helps, too, to shade the older ones that are more susceptible to abnormal evaporation.

SUMMARY

1. The potato plant early in the season records the highest osmotic pressure in the sap from the young stalks and leaves.

2. During the very hot weather of July and August, the sap of the stalks develops a higher osmotic pressure than that from the younger portions of the plant.

3. The high pressure in the stalks is due to the presence in them of sugars, especially of cane sugar.

4. In September, after growth has been resumed, the young leaves again have the highest osmotic pressure of any portion of the plant.

5. The osmotic pressure of the sap of the growing tubers is always low and is intermediate between that of the sap of the stalk and the sap of the roots, which latter is the lowest of all.

6. The osmotic pressure in the older plants is higher than that in the younger ones and is due to the larger amounts of inorganic salts in the former. In very old plants, however, the soluble materials are removed to a considerable extent, and the osmotic pressure of the sap drops as a consequence.

VERMONT AGRICULTURAL EXPERIMENT STATION.

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CYRTANDREAE HAWAIIENSES, SECT. MICROCALYCES HILLEBR.

Joseph F. Rock

The present paper concludes the monograph of the Hawaiian representatives of Cyrtandra,[1] which number ninety-five species, varieties, and forms. The Hawaiian species were divided by Hillebrand into five more or less well-defined sections as follows: Cylindrocalyces, Crotonocalyces, Schizocalyces, Chaetocalyces, and Microcalyces. Hillebrand's system has been here adhered to rather than that of C. B. Clarke, who includes foreign species with Hawaiian species in some of his sections. The latter arrangement does not seem to be a satisfactory one, for what must be recognized as a variety of a species is to be found in a different section from that to which the species is referred. Krauflein in his paper on the Philippine Cyrtandreae says that C. B. Clarke's arrangement is for the present still quite satisfactory, which of course may be true for the Philippine and Malayan species, but certainly not for the Hawaiian species. The writer has described sixteen new species, twenty-one new varieties, and six new forms, all of which save one (*C. cyaneoides*) are described in the present series of papers.

Through the courtesies extended the writer by the directors of the various herbaria in Europe where he worked shortly before the outbreak of the recent war, and through the loan of material from the Gray Herbarium and the Cornell Herbarium, the writer was in a position to unravel the existing confusion in this difficult group of plants. The writer is especially indebted to Prof. B. L. Robinson of Harvard and to Dr. Rowlee and Prof. Hosnier of Cornell for the loan of material at a time when transportation facilities are more or less upset and when the shipping of types is exceedingly hazardous.

That there remain additional new species of Cyrtandreae to be discovered in Hawaii there is no doubt; especially will Kauai yield a goodly number from the little explored gorges on the windward side. Cyrtandreae, like the Hawaiian Lobelioideae, are very local, and it is therefore to be expected that the numerous deep ravines on the windward side of Oahu and the other islands will furnish new species; the Punaluu region on Oahu is a veritable paradise for the botanist and as splendid a collecting ground as is to be found anywhere in this group of islands. Cyrtandreae are especially abundant there in the deep shade along the numerous watercourses.

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[1] For the previous papers of the series, see Amer. Journ. Bot. 4: 604–623. 1917. Ibid. 5: 259–277. 1918. Ibid. 6: 47–68. 1919.

Unfortunately the writer was compelled to write an addendum in which there are described species and varieties belonging to the section Cylindrocalyces.

He would also call attention to *C. Pickeringii*, the synonymy of which is thoroughly discussed under *Cyrtandra Oliveri* in the preceding paper. The task of arranging these difficult plants was not an easy one, and the writer would refer the reader to the introductory remarks in the first paper of the series, so that he may appreciate the difficulties encountered.

SECTION FIVE: MICROCALYCES CINEREAE Hillebr. Fl. Haw. Isl. 326. 1888

Calyx small, five-fid to the middle into narrow lobes. Corolla erect with small lobes. Flowers many in open cymes. Leaves broad, obovate or elliptical. Tomentum, when present, short-cinereous or pale ochraceous.

The species of this section are characterized by the small calyx and small calycine lobes; they certainly form a distinct group, though some are related to species of the section Crotonocalyces, as for example *Cyrtandra Garnotiana* to *C. honolulensis*. *Cyrtandra triflora* is placed in this section but with some doubt, as the writer has been unable to examine the type. The other species of this section are *C. laciflora* and *C. polyantha*. A single new species belonging to this section has been found on Hawaii; it is a small tree fifteen feet or more in height, and grows in the vicinity of the Volcano of Kilauea in the fern forests, but especially in Mr. W. M. Giffard's forest residence, Kalauilehua. It has been named after Mr. Giffard (*Cyrtandra Giffardii*), who recently collected splendid specimens of Cyrtandreae on Hawaii.

CYRTANDRA TRIFLORA Gaud. Bot. Voy. Uranie 447, t. 52. 1826

"Foliis oblongis aut ellipticis, breviter acuminatis, basi cuneatis, leviter serratis, supra glabris, subtus secus nervos adpresso-pubescentibus; pedunculis trifloris; calyce glabriusculo, dentibus ovato acuminatis tubo brevioribus vel cum hoc vix aequilongis. G. Don Gen. Syst. 4, p. 661. neque Hooker et Arn., neque Asa Gray.

"Folia opposita subaequalia, longa 10–6 cm., lata 4 cm., nervi primarii laterales utrinque 8–9; petioli 5 cm.; pedunculi 1–2 cm.; fusco pubescentes; bracteae 8 mm., oblongae. Calyx 11 mm. longus, minute furfurascens; tubo 6 mm., cylindrico-campanulaceus. Corolla longa 22–25 mm., fere recta, extus superne rufescenti-villosula."

INS. SANDWICH: Gaudichaud in herb. De Caudolle, Paris.

Unfortunately the writer has been unable to examine the type of this species. It seems that a number of botanists were unable to place this species, and it seems too that no one has re-collected it since Gaudichaud's first visit. C. B. Clarke saw the type and states that neither Hooker and Arnott's nor Asa Gray's specimens designated as *C. triflora* are referable to that species. Hillebrand's specimens referred to that species were described by C. B. Clarke as *Cyrtandra polyantha*. As can be judged from the description, which is here copied from C. B. Clarke, the species belongs undoubtedly to this section. Asa Gray's specimen referred by him to *Cyrtandra triflora* is a variety of *C. platyphylla* and must be referred to the writer's *C. platyphylla parviflora*. The drawing in the Atlas (Bot. Voy. Uranie) is too schematic to permit identification.

Cyrtandra Giffardii Rock n. sp.

A small, soft-wooded tree 5 m. in height, with numerous tortuose thin branches, the latter quadrangular and pubescent near their apices with dark blackish-brown hairs; leaves elliptical or obovate, chartaceous, dark green, acute at the apex, cuneate at the base, with denticulate margin, subglabrate above or with single scattered hairs, the midrib and veins prominent underneath, and covered with dark brown hair, 6–8 cm. long, 2.5–3.5 cm. wide, on dark brown pubescent petioles of 2–2.5 cm.; inflorescence a three-to four- or five-flowered cyme, covered with a dark brown tomentum throughout; peduncle 2–3.5 cm. long, slender; pedicels 2–3 cm. long, slender, the bracts linear-lanceolate, 6–7 mm. long; calyx tube short, 2–3 mm., the linear acuminate lobes 4–5 mm., hairy outside, glabrate inside; corolla small, 12 mm. long, constricted at the throat, straight, hirtulose; berry small, globose to ovate.

HAWAII: Forests near the Volcano House, especially Kalauilehua, fruiting July, 1911, Rock no. 10337 in herb. College of Hawaii; same locality, flowering January, 1918, W. M. Giffard (type) no. 13096 in herb. College of Hawaii.

A very distinct species probably distantly related to *Cyrtandra polyantha;* it is a small tree fifteen feet or so in height and is much branched with numerous small tortuose branchlets. It is named for Mr. W. M. Giffard who collected flowering material of this plant.

CYRTANDRA GARNOTIANA Gaud. Bot. Voy. Uranie 447, t. 53. 1826

Cyrtandra Carnotiana G. Don. Gen. Syst. **4**: 661. 1838.
Cyrtandra Vaniotii Lév. Repert. Sp. Nov. Fedde **10**: 155. 1912.

Branches obscurely triangular, the young shoots canescent-tomentose; leaves opposite, subequal, up to 18 cm. long, 10 cm. broad, acute, attenuate or rounded at the base, chartaceous, puberulous above, gray-tomentose and pale underneath, the margin denticulate or serrulate; cyme 5–10 cm. long, densely but shortly canescent-tomentose, 5–20-flowered; common peduncle 2.5–5 cm., stout, bracteate at the apex, the bracts ovate-acute, clasping at the base, 1 cm.; pedicels 1.5 cm.; calyx gray-tomentose, 8 mm. long, divided to the middle into five triangular-oblong lobes; corolla small, 8–16 mm., straight, tubular, widening below, with nearly equal lobes; ovary and style pubescent; berry ovoid, 14–16 mm. long, acute.

OAHU: Gaudichaud in Gray Herbarium; U. S. Explor. Exped. in Gray Herbarium; Mann and Brigham no. 126 in Gray Herbarium, no. 77 in Cornell Herbarium; Hillebrand, western part of the island, in herb. Berlin; Hillebrand, Wahiawa, in herb. College of Hawaii; R. S. Hosmer, Mt. Kaala, no. 13088 in herb. College of Hawaii; U. Faurie, Kaala Mts., November 1909, no. 1144 (labeled *C. Wainotii*) in herb. College of Hawaii.

Specimens examined in the herbaria of Europe: In Museum Botanicum Berolinense:—

Cum determinat. C. B. Clarke	Ex herbario Gaudichaud, a small branchlet with fruit marked "Gaudichaud ded. 1829, Sandwich." It bears C. B. Clarke's determination as *C. Garnotiana.* A second sheet, no. 551, coll. Gaud., visit 1841. det. C. B. Clarke. Ex herbario Hillebrand; Oahu, western part of the island.
Sine determinat. C. B. Clarke	Ex herbario Wawra, Erdumseglung Donau 1868–1871. Specimen with flower bud and fruit. Ex herbario Hillebrand, from Wahiawa, Oahu, with young flowers. Another sheet with large leaves from Mt. Kaala, Oahu, July, 1869, Hillebrand.

In herbario Caesareo Palat. Vindobon. (Wien) Vienna.

Ex collect. Wawra, Oahu, no. 1981, with C. B. Clarke's determination.

Another sheet, same number (1981), with large leaves, without C. B. Clarde's determination.

C. B. Clarke's citation of species: Gaudichaud in herb. De Candolle, Berlin, Delessert; Seemann no. 1277 in herb. De Candolle; Hinds, in herb. Kew; Hillebrand no. 321 in herb. Kew et Berlin; Wawra no. 1981 in herb. (Wien) Vienna; Beechey in herb. Kew, Delessert; Seemann no. 1722 in herb. De Candolle et Kew.

Cyrtandra Garnotiana is evidently confined to the western end of Oahu, especially Mount Kaala. U. Faurie collected a small-leaved form of it which was described by Léveillé as *C. Vaniotii.* The specimens are however labeled *C. Wainotii;* the number cited by Léveillé (no. 1144) is the same as on Faurie's specimen with the latter spelling. His specimen labeled *C. Garnotiana* no. 637 Faurie, is not referable to that species, as can be seen from the shape of the leaves, but as it is without flower or fruit must remain unidentified. It belongs however to the group with *C. Pickeringii.*

C. B. Clarke describes a variety β (*fulva foliis subtus magis fulvis; calyce altius diviso*). Ins. Sandwich; Oahu, Barclay, in herb. British Museum. The writer has not seen this plant, but it evidently is only a form of *C. Garnotiana* Gaud.

CYRTANDRA LAXIFLORA Mann, Proc. Amer. Acad. 1: 190. 1868

Branches scarcely quadrangular, glabrous; leaves 15–20 cm. long, 6–13 cm. wide, acute at the apex, unequally obtuse at the base, rarely rhomboidal, oblong to ovate, serrulate, with scattered multicellular hairs above, softly yellowish-pubescent below; petioles 3–8 cm.; cymes lax, twice dichotomously branching; peduncle 5 cm.; bracts 1–2 cm., oblong to linear spathulate; pedicels 1–2 cm., villous; calyx minutely villous, with five oblong-linear lobes, 6–11 mm. long; corolla 2 cm., narrow, cylindrical, hirsute to villous outside; ovary almost glabrous; stigma after anthesis bilobed; fruit slightly villous at the apex as is also the short style, the latter slightly exceeding the calyx.

OAHU: Waialua mountains, Mann and Brigham no. 615 in herb. Kew, herb. Cornell University, and Gray Herbarium; Waianae range, Hillebrand in herb. Berlin and part (fragment) in herb. College of Hawaii.

Cyrtandra laxiflora Mann is certainly a distinct species and, as Mann states, comes close to *C. Macraei* but in flowers only.

To *C. laxiflora* Mann must be referred as a special variety Hillebrand's var. *grandifolia* of *C. triflora* Hillebr. not Gaud. This latter plant is certainly much more closely related to *C. laxiflora* than to *C. polyantha* C. B. Clarke (*C. triflora* Hillebr.). Here belongs also a new variety from Kaliuwaa Valley, *C. laxiflora rhyzantha*, which see.

CYRTANDRA LAXIFLORA **rhizantha** Rock n. var.

A shrub with few ascending branches; leaves 15–20 cm. long, 6.5–8 cm. wide on petioles of 4–10 cm., sharply dentate, otherwise as in *Cyrtandra laxiflora* var. *grandifolia;* inflorescence on the stem near the ground and on exposed roots, a twice to thrice dichotomous cyme, the peduncle of varying length, 1.5–3 cm. long; bracts 8 mm.; ultimate pedicels of varying length, villous; calyx and corolla as in var. *grandifolia.*

OAHU: Koolau Mts., Punaluu, flowering October 31, 1914, Rock no. 13086 in herb. College of Hawaii.

This plant differs little from *C. laxiflora* var. *grandifolia,* but mainly in the smaller leaves, and open and lax thrice-dichotomously branching cyme. The inflorescence is borne on the lower portions of the stem and on exposed roots, hence the name.

CYRTANDRA LAXIFLORA **grandifolia** Rock n. comb.

Cyrtandra triflora Hillebr. var. *grandifolia* Hillebr. Fl. Haw. Isl. 332. 1888.

Branches glabrous, apices hirtellous to villous; leaves large, ovate to suborbiculate, 15–24 cm. long, 6.5–14 cm. wide, acuminate at the apex, rounded at the base or uneven-sided, hispidulous above, pubescent below, especially on the stout midrib and nerves; petioles 3–5.5 cm.; cymes large, open, branching; bracts 7 mm., acute; pedicels filiform, villous; calyx as in *C. laxiflora,* more deeply divided, the lobes linear-acute, 8 mm.; corolla 12–14 mm., the tube narrow-cylindrical, ampliate at the throat, hirsute especially in the upper third; ovary elliptical, sessile and rounded at the base, long acuminate at the apex, puberulous; fruit twice as long as the calyx, elliptical-acuminate, puberulous.

OAHU: Waialua and Waipio, Hillebrand in herb. Berlin and part of type in herb. College of Hawaii; mountains of Waialua, flowering February 10, 1907, Otto. H. Swezey no. 12773 in herb. College of Hawaii.

According to the right interpretation of *Cyrtandra triflora* Gaud. and *C. polyantha* C. B. Clarke, Hillebrand's variety *grandifolia* of his *C. triflora* cannot be retained, but must be referred as a variety to *Cyrtandra laxiflora.* Even should Hillebrand's determination or interpretation of Gaudichaud's *C. triflora* have been correct, it could not possibly have been retained as a variety of *C. triflora* Gaud., but would have had to be referred to *C. laxiflora* to which it comes so close as to make even its varietal rank questionable. It differs from *C. laxiflora* mainly in the smaller flowers and perhaps larger leaves.

CYRTANDRA POLYANTHA C. B. Clarke in DC. Monogr. Phan. 5: 220. 1883–1887.

Cyrtandra triflora Hillebr. Fl. Haw. Isl. 332. 1888, in part, not Gaud.
Cyrtandra gracilis Drake Cast. Illustr. Fl. Ins. Mar. Pacif. 7: 253. 1892, not Hillebr.

A small shrub, 1 m. high; branches subterete, glabrous, young portions yellowish-villous to woolly; leaves opposite, of equal size, 12 cm. long, 2.5–3.5 cm. wide according to type (5–7 cm. broad *teste* C. B. Clarke), closely denticulate, elliptical, acuminate at both ends, coriaceous, slightly scabrous above, shortly yellowish-silky beneath; cyme dichotomously branching, densely many-flowered; peduncle 5–8 mm., almost glabrous, bracts 6 mm., lanceolate; pedicels up to 8 mm., glabrate; calyx divided to the middle, the lobes 2 to 3 mm.; corolla 12 mm., cylindrical, hirsute or villous; ovary glabrous, the style partly glandular-pilose.

OAHU: Mountains of Ewa, Hillebrand in herb. Kew, Berlin, and Gray Herbarium, clastotype in herb. College of Hawaii; main ridge of Niu Valley, elevation 1700 feet, flowering August 22, 1909, Rock no. 4815 in herb. College of Hawaii.

The specimen in the Hillebrand Herbarium in the Berlin Botanical Museum bears in C. B. Clarke's handwriting the name *C. polyantha* C. B. Clarke, and in Hillebrand's handwriting the name *C. triflora*, Oahu, Mts. of Ewa.

Drake Del Castillo is absolutely wrong in citing *C. polyantha* as a synonym of *C. gracilis;* the plant has nothing in common with it. In *Cyrtandra gracilis* the calycine lobes are very long and subulate, and the whole aspect of the plant is different; besides the leaves in *C. polyantha* are densely silky-villous below.

The writer's specimens came from an exposed ridge or spur in Niu Valley and agree quite well with the specimen marked as *C. polyantha* by C. B. Clarke in the Berlin Herbarium, with the exception of the peduncles which are up to 2 cm. in length. There is, however, no doubt that it is *C. polyantha.*

CYRTANDRA POLYANTHA **ambigua** Rock n. comb.

Cyrtandra triflora Hillebr., var. γ *ambigua* Hillebr. Fl. Haw. Isl. 332. 1888.

Leaves 8 cm. long, 2.5 cm. wide, acuminate at both ends, closely denticulate, coriaceous, on petioles of 2 cm.; peduncle 8 mm., pedicels several of variable length, glabrous as is the calyx; calyx one-half or two-thirds the length of the corolla, cylindrical, shortly and unevenly divided to less than the middle into lanceolate-acute lobes or teeth, beaked in the bud and splitting laterally; corolla cylindrical, hirsute, the lobes small.

OAHU: Hillebrand in herb. Berlin and herb. College of Hawaii.

Hillebrand's var. *ambigua* of his *C. triflora* is exceedingly close to *C. polyantha* and almost identical with it, but still worthy of varietal rank. As it does not belong to *C. triflora* Gaud. but to *C. polyantha* C. B. Clarke, it is here referred to it as a variety. Hillebrand gives no other locality than Oahu. The writer has not collected this variety.

ADDENDA SECTION CYLINDROCALYCES

Cyrtandra limosiflora Rock n. sp.

Single-stemmed, bearing a crown of leaves at the apex, 1–1.5 m. high; stem stout, 5 cm. in diameter near the base, distinctly nodose, the nodes at intervals of 2.5–3 cm., greenish-gray; leaves large, quaternate, sessile, forming a large rosette-like crown, chartaceous, broadly lanceolate-oblong, acuminate at the apex, gradually tapering from the middle of the leaf to a broad sessile base, 45–50 cm. long, 10–12 cm. wide (2–2.5 cm. wide at the sessile base), the margin denticulate, sparingly so towards the base, dark green above, pale grayish-brown beneath, glabrous above, puberulous to glabrous below but somewhat pubescent along the midrib and veins; inflorescences densely crowded around the stem for a length of 14–20 cm., surrounding the stem at each node and in the axils of the leaves; peduncle 3–5 mm., bearing about ten flowers; pedicels about 4 mm.; calyx pale green, cylindrical, puberulous outside, densely hirsute with long hairs inside, about 2.5 cm. long, bilabiate, unequally deeply slit into four lobes with long filiform apices; corolla the length of the calyx, slightly curved, glabrous throughout, the lobes unequal, oblong, rounded; style glabrous, the stigmatic lobes large, ovate-oblong; fruit ovoid-oblong, glabrous.

MOLOKAI: Mapulehu Valley, Pukoo, among rocks in stream bed, flowering December 24, 1915, Rock no. 12522, type in College of Hawaii Herbarium.

A remarkable species, resembling in habit a Cyanea. The name *limosiflora* refers to the slimy inflorescence which is usually full of insect life, slugs, and decayed vegetable matter and gelatinous substances. It is related to *Cyrtandra longifolia* var. *degenerans* C. B. Clarke, but differs from it in the very large, broadly sessile leaves and numerous inflorescences which extend down the stem for sometimes 20 cm. It belongs to the section Cylindrocalyces, from which it was omitted by an oversight.

In Halawa Valley on the same island (Molokai) there occurs a Cyrtandra with very large leaves which resemble those of *C. limosiflora* but are distinctly oblong and do not taper towards the base from the middle of the leaf but from the lower third; they are 60–70 cm. long and about 15 cm. wide, and puberulous on both faces, the petiole none. Unfortunately the plant was neither in flower nor in fruit. The leaf specimens were collected in April, 1909, in Halawa Valley; they are deposited in the College of Hawaii Herbarium and bear the number 13101. There is no doubt that these specimens represent an undescribed species of the section Cylindrocalyces as can be judged by the habit of the plant.

CYRTANDRA PALUDOSA BREVICALYX Hillebr. forma **linearis** Rock, n. f.

Cyrtandra paludosa var. *filipes* Hillebr. ms. in Gray Herbarium.

A shrub; branches terete, glabrous; leaves linear-lanceolate, elliptical, 7–11 cm. long, 1.5–2.5 cm. wide, acuminate at both ends, the margin wavy-crenate, chartaceous, pale green and glabrous on both sides; petioles 2–3.5 cm.; peduncle short, 2–5 mm., bearing single flowers on pedicels of 2.5–3 cm.; fruit elliptical-fusiform.

OAHU: Kaala Mts., Hillebrand, in Gray Herbarium; eastern range (Kaala) Schofield Barracks, flowering July 11, 1916, A. S. Hitchcock no. 14027 in U. S. National Herbarium and clastotype in College of Hawaii Herbarium.

This form differs from the variety *brevicalyx* mainly in the linear-lanceolate leaves. The specimen in the Gray Herbarium *ex coll.* Hillebrand is labeled *C. paludosa* var. *filipes.*

CYRTANDRA CONFERTIFLORA C. B. Clarke in DC. Monogr. Phan. **5**: 235. 1887

Cyrtandra paludosa Gaud. var. γ *confertiflora* Wawra, Flora **30**: 560. 1872.

Branches thick, subquadrangular, the apex fulvo-sericeous; leaves large, opposite, elliptical, serrate, 28 cm. long, 12 cm. broad, acute at both ends, silky-villose along the nerves below; petioles 5–8 cm., narrowly winged cymes subsessile, almost capitate, yellowish-silky when young; bracts 2 cm., glabrate, deciduous; calyx 12 mm., subovate, rostrate and closed before flowering, reddish yellow-silky, finally glabrate and deciduous; corolla 25 mm. long, the upper lip erect, the lower divaricately opened, almost glabrous; fruit 1 cm. long, 7 mm. broad, ovoid, subconical.

KAUAI: Dense forest of Kealia, Wawra no. 2057 in herb. Vienna; U. Faurie, December, 1909, no. 618, in herb. College of Hawaii, no. 13098.

Cyrtandra confertiflora C. B. Clarke is a decidedly distinct species and differs from *C. paludosa* mainly in the larger leaves and the densely glomerate cyme practically without peduncle and with short pedicels. The writer has examined the type in the Vienna Herbarium, but through some error he omitted it from the section Cylindrocalyces where it belongs. U. Faurie's specimens of this species were identified by Hector Léveillé as *Cyrtandra latebrosa* Hillebr. and distributed as such.

CYRTANDRA LONGIFOLIA Hillebr.; C. B. Clarke in DC. Monogr. Phan. 5: 276. 1883–87

Cyrtandra paludosa Gaud. var. *longifolia* Wawra, Flora 55: 558. 1872.
Cyrtandra paludosa Lév. (not Gaud.) ms.

U. Faurie, who collected the typical *Cyrtandra longifolia*, distributed it (no. 610) as *Cyrtandra paludosa* Gaud.; the plants were identified by H. Léveillé.

A. A. Heller collected specimens of a Cyrtandra, no. 2624, which he distributed to various herbaria as *Cyrtandra longifolia* and *Cyrtandra Wahiawae* n.sp. The former is in the herbarium of Cornell University and the latter in the Gray Herbarium; both bear the number 2624 and are identical. The plant is not referable to *Cyrtandra longifolia*, nor can it very well be retained as a new species as it is not sufficiently distinct from the former, but may best be disposed of as *Cyrtandra longifolia Wahiawae* (Heller) Rock. The plant is entirely glabrous throughout save the juvenile leaves; the leaves are smaller than in the species and are more distinctly petiolate.

CYRTANDRA LONGIFOLIA γ PARALLELA C. B. Clarke in DC. Monogr. Phan. 5: 277. 1883–1887

"Foliis oblongis, lateribus subparallelis; petiolis usque ad 5 cm., longis, minus alatis. *C. longifolia typica* Hillebrand ms."

INS. SANDWICH. Hillebrand no. 327 in herb. Kew.

This variety is too briefly described to enable any one to identify it. No locality is given other than Sandwich Islands. Hillebrand's specimens collected later do not bear the same names as those which he previously sent to Kew and Berlin; besides, none of the Berlin material *ex herb.* Hillebrand bears numbers. The var. γ *parallela* is not known to the writer. A. S. Hitchcock collected a Cyrtandra, no. 15446 on Kauai, Waialeale, which may be referable to this variety of *C. longifolia.* That it belongs to one of the many varieties of *C. longifolia* there is no doubt. The inflorescence is cymose and the peduncle about 1 cm. long, otherwise it could be referred to *C. longifolia* var. *arborescens* (Wawra) C. B. Clarke. It may also be a form of *C. paludosa* var. *Gayana* (Heller) Rock. The specimen is too fragmentary to allow a definite diagnosis (no. 15446 in U. S. National Herbarium).

CYRTANDRA HAWAIIENSIS C. B. Clarke in DC. Monogr. Phan. 5: 235. 1883–1887

Cyrtandra paludosa var. *integrifolia* Hillebr. (in part) Fl. Haw. Isl. 337. 1888.
Cyrtandra longifolia Drake Cast. Fl. Ins. Mar. Pacif. 7: 256. 1892, not C. B. Clarke.

"Foliis magnis sessilibus, spathulato-oblongis, subintegris, maturis fere glabratis; calycibus 2–3 cm., semi-5-fidis, lobis lanceolato-linearibus. Ramuli teretes, crassi; novellae partes fulvo-villosae. Folia longa 3 dm., lata 7 cm., supra glabrata, subtus secus nervos fulvide pubescentia, nervi primarii laterales utrinque 12. Cymae axillares, pluriflorae, subcapitatim condensatae. Calyx viridis, angustus, extus glabriusculus, intus fulvo-hirsutus. Corolla extus glabra. Stamina 2, glabra; antherarum loculi oblongi, paralleli, rimis apice curvatis, mox in unam confluentitus. Ovarium cum stylo glabrum; discus brevis annularis. Bacca non visa. Exemplum mancum; folia non certe opposita."

INS. SANDWICH; HAWAII: Kohala, Hillebrand no. 333 in herb. Kew.

This is the plant the writer mentioned under the *Specimina excludenda* of *Cyrtandra paludosa*, stating on page 611 of this publication (Amer. Journ. Bot. December, 1917): "Hillebrand's specimen from the Kohala Mts. Hawaii and referred by him to the above variety [*C. paludosa* var. *integrifolia*], with Knudsen's no. 137, is an entirely different plant and has absolutely nothing in common with *C. paludosa* or with *C. longifolia;* the leaves remind one very much of a species of Shorea. It represents an undescribed species."

The specimen in the Berlin Herbarium (without number) is without flowers and possesses only a couple of old broken-up fruits from which the calyx has disappeared. Through cross references and the description the writer finally traced this specimen to C. B. Clarke's *C. hawaiiensis* from the Kohala Mountains. It certainly represents a different species, and in his notes on Cyrtandra made in Berlin, the writer suggested the name *shoreaefolia;* if Hillebrand had given numbers to his own specimens, as he did to those of his he sent to Kew, matters might have been less confusing.

CYRTANDRA MACRANTHA C. B. Clarke in DC. Monogr. Phan. 5 265. 1883–1887

"Foliis magnis petiolatis, oblongo-ellipticis subintegris, maturis fere glabratis; calyce 4 cm., lobis ovato-lanceolatis; corolla 6–7 cm.; frutex 10-petalis; ramus crassus; novellae partes minute tomentellae, pilis ferrugineis parce additis. Folia longa 3–4 dm., lata 12 cm., basi cuneata; nervus medius supra prope basin fulvide hirsutus; nervi primarii laterales utrinque 14; petioli 3 cm. Flores axillares condensati. Calyx tubulosus sub anthesis usque ad tertiam partem divisus, extus subglaber, intus fulvide tomentellus, lobis caudatis. Corolla tubulosa, leviter caudata, extus glabra; lobi 2 cm., rotundati. Bacca non visa."

INS. SANDWICH: in vallibus a mare remotis (herb. Hooker) sine nomine lectoris in herb. Kew.

If this is really a Hawaiian species, it certainly possess the largest flowers of any of our Cyrtandreae. The description of the calyx places this species at once in the section Cylindrocalyces. It is evidently closely related to the writer's *Cyrtandra waianuensis*, from which it differs in the exceedingly large flowers and the 3-cm.-long petiole.

CYRTANDRA GLAUCA Drake Cast. Fl. Ins. Mar. Pacif. 7: 253. 1892

"Glaberrima, foliis membranaceis, elliptico-lanceolatis (15–18 cm. longis, 3–4 cm. latis, petiolo 2–3 cm. longo) grosse serratis. Flores solitarii, pedunculo gracili petiolum aequante, calyce oblongo (1 cent.) fere usque ad dimidiam partem fisso, corollae tubo brevi, fauce leviter ampliata, bacca oblonga."

KAUAI: Remy no. 440 bis, herb Paris (?)

The writer has not seen this species and he can only quote here the description. It probably belongs to the section Cylindrocalyces as can be judged from the description of the calyx.

CYRTANDRA SCABRELLA C. B. Clarke in DC. Monogr. Phan. 5: 277. 1883–1887

"Scabrella; foliis ellipticis utrinque acutis, calloso serratis, cymis 3-floris; calyce juniore usque ad mediam partem diviso, lobis ovato-oblongis. Rami vix quadrangulares. Folis opposita subaequalia, longa 1 dm., lata 4 cm., in utraque superficie (supra parcius) a pilis fulvis minutis scabra; nervi primarii laterales utrinque 6, subtus elevati; petiolus 2 cm. Pedunculus 12 mm., bracteae 12 mm.; ellipticae, subacutae; pedicelli 12 mm. Calyx sub anthesi longus 2 cm., lobi 11 mm., minute scabrelli. Corolla 25 mm.; tubus paullo curvatus, extus villosus."

INS. SANDWICH: Hillebrand no. 324 in herb. Kew.

The writer has not seen this species, and even from the description is unable to give an opinion as to the validity of the species. It may be related to *C. longifolia*, but the "callous serrate leaves" would bring it closer to *C. paludosa* than to the former, which has entire leaves. C. B. Clarke's description is here quoted.

CYRTANDRA OENOBARBA Mann.

Horace Mann included in his *C. oenobarba* from the Wahiawa Falls, Kauai, no. 616, specimens also numbered 616, but which are not identical with the type of *C. oenobarba*. Wawra collected the same plant which Wawra evidently erroneously placed with *C. oenobarba* and described it as *Cyrtandra paludosa* var. *herbacea*. Heller, who also collected the latter plant, recognized correctly that the plant is not so much related to *C. paludosa* as to *C. oenobarba* and correctly changed it to *Cyrtandra oenobarba* Mann var. *herbacea* (Wawra) Heller. Mann's specimens no. 616 are only *pro parte* referable to *C. oenobarba*. The species is distinguished by the shaggy hairs with which the leaves and petioles are covered, while the variety *herbacea* is glabrous.

The writer had all specimens herein mentioned at his disposal for comparison.

Cyrtandra montis Loa Rock n. sp.

A shrub 3–5 m. high with few ascending branches, the latter terete; bark thin, grayish-brown, glossy, glabrous, excepting the apex which is covered with dirty brown hair; leaves ternate, thick-coriaceous, elliptical-oblong, acute at the base, acuminate at the apex, dark green and glossy above, the veins impressed, pale dull green beneath with the hispidulous midrib and veins very prominently projecting, the margins irregularly and coarsely serrate, excepting the entire base, 20–25 cm. long, 6–9 cm. wide, on petioles of 6–9 cm.; cymes axillary in the five upper leaf-whorls, 3–7-flowered; peduncle thick, fleshy, 1–2 cm. long, bearing at the apex two large foliaceous bracts, sessile and subcordate at the base, up to 4 cm. long, 2 cm. wide, 3–5-nerved, peduncle and pedicels hirsute with dark brown hair, the pedicels of varying length up to 3.5 cm. when with fruit; calyx large, divided to near the base into five large foliaceous lobes, which are distinctly 3-nerved; the lobes ovate, acute at the apex, 2.5 cm. long, 1.5 cm. broad near the base; corolla hidden in the calyx, the tube straight or slightly curved, constricted at the throat and hairy in the upper portion, the lobes small, not spreading, and subequal; style short, curved, the broadly bilamellate stigma downward-spreading; ovary glabrous; fruit large, obovate, 2 cm. long, 18 mm. thick, obtuse but crowned with the remnant of the style.

HAWAII: Not uncommon in the forests on the northeastern slope of Mauna Loa, especially in the dense tree-fern forests between 29 miles and Kulani (neighborhood of the Volcano of Kilauea), elevation 3000–5000 feet, in company with *Cyrtandra platyphylla typica*, *Cibotium Menziesii*, *C. Chamissoi*, *Pritchardia Beccariana*, *Cyanea pilosa*, etc., flowering and fruiting August 15, 1918, Rock and Hashimoto no. 13115 (type) in herb. College of Hawaii.

Cyrtandra montis Loa, confined to the fern forests of the northeast slope of Mauna Loa, is a very distinct species and easily recognized by its terete stems, and large, leathery, deep green leaves with impressed veins. The tallest specimens the writer observed were near the summit of the ancient cone crater Kulani, 5500 feet elevation. It is the predominating species in the region of Kilauea.

The species belongs to the section Schizocalyces Hillebr., but on comparison with the other species of that section we find no close relationship with any of them. Like *C. umbracculiflora* it is a very distinct species with no apparent relationship to the rest of the species of that section, but with leanings towards species in the section Crotonocalyces.

Cyrtandra ramosissima Rock n. sp.

A tree 5 m. high or more with a trunk about 15 cm. in diameter; bark smooth, usually covered with mosses and ferns; branches many, tortuose, the branchlets twisted, terete, and nodose, the nodes at close proximity to each other towards the apex; leaves opposite, pale green above, paler to whitish below, the veins impressed above, and prominently projecting below, hirsute with pale brownish hair above, shortly pubescent to puberulous below, thin, submembranous, with pellucid veins and nerves, elliptical, acute at the base, acuminate at the apex, coarsely and irregularly serrate with the exception of the lower fourth which is entire, 10–12 cm. long, 5.5 cm. wide, on hirsute petioles of 2.5–4 cm.; flowers solitary in the axils of the upper leaves on a hirsute peduncle of 12–15 mm. in length, bracteate at the apex, the bracts lanceolate-foliaceous, hirsute, distinctly 3-nerved, 2 cm. long, 6 mm. wide; pedicel of the same length as the peduncle; calyx deeply divided to the lower third into five lanceolate, hirsute, 1-nerved, acute lobes, 8–10 mm. long, 2.5–3 mm. wide; corolla white, slightly exceeding the calyx, semi-erect or slightly curved, widening at the throat, hirtellous in the upper portion, the lobes of equal size, small and rounded; ovary elliptical, 4 mm. high, white, glabrous as is the short style; stigma broadly two-lobed, the lobes elliptical; anthers slightly protruding from the throat; fruit obovate to elliptical, glabrous, crowned by the short style.

HAWAII: In dense rain forest near Glenwood, elevation 2400 feet, rarely ascending higher, flowering and fruiting August 19, 1918, Rock and Hashimoto no. 13116 (type) in herb. College of Hawaii.

This species and *Cyrtandra Giffardii* are then apparently the only arboreous Cyrtandreae in the Hawaiian Islands. It is a many-branched small tree 15–20 feet in height with a single woody trunk over five inches in diameter. It belongs to the section Schizocalyces Hillebr. and is related to *Cyrtandra lysiosepala* (Gray) Clarke, from which it differs in the single-flowered inflorescence, the small corolla lobes, the hirsute leaves and green calyx and bracts, which are white in *C. lysiosepala*.

Cyrtandra Hashimotoi Rock n. sp.

A much-branched, tortuose shrub 1–1.5 m. high, the branches rough and twisted, ultimate branchlets terete, nodose, and glabrous, with the exception of the apex, this coarsely hirsute with short, stiff, brownish-black hairs; leaves opposite, small, elliptical to obovoid, acuminate at the apex, acute at the base, 5.5–6.5 cm. long, 1.75–2.5 cm. wide, thick-coriaceous, dark green above, brownish beneath, with scattered stiff, reddish-brown hairs on the upper surface, glabrous on the lower, with the exception of the very prominently projecting midrib and veins which are hirsute; petioles hirsute, 1–2 cm. long; flowers single in the axils of the upper leaves; peduncle 10–12 mm. long, hirsute with brownish hairs; bracts or bractlets none; calyx divided to the base into linear-subulate lobes, these hirsute outside and subglabrous inside, 1 cm. long, less than 1 mm. wide, acute, and broader at the base; corolla nearly twice as long as the calyx, the tube narrow-cylindrical, curved, 2 mm. wide, strongly hirsute outside with the exception of the lobes which are subglabrous, rounded, small, equal in size and not spreading; ovary turbinate, puberulous or glabrous, the acute apex crowned by the short style; fruit unknown.

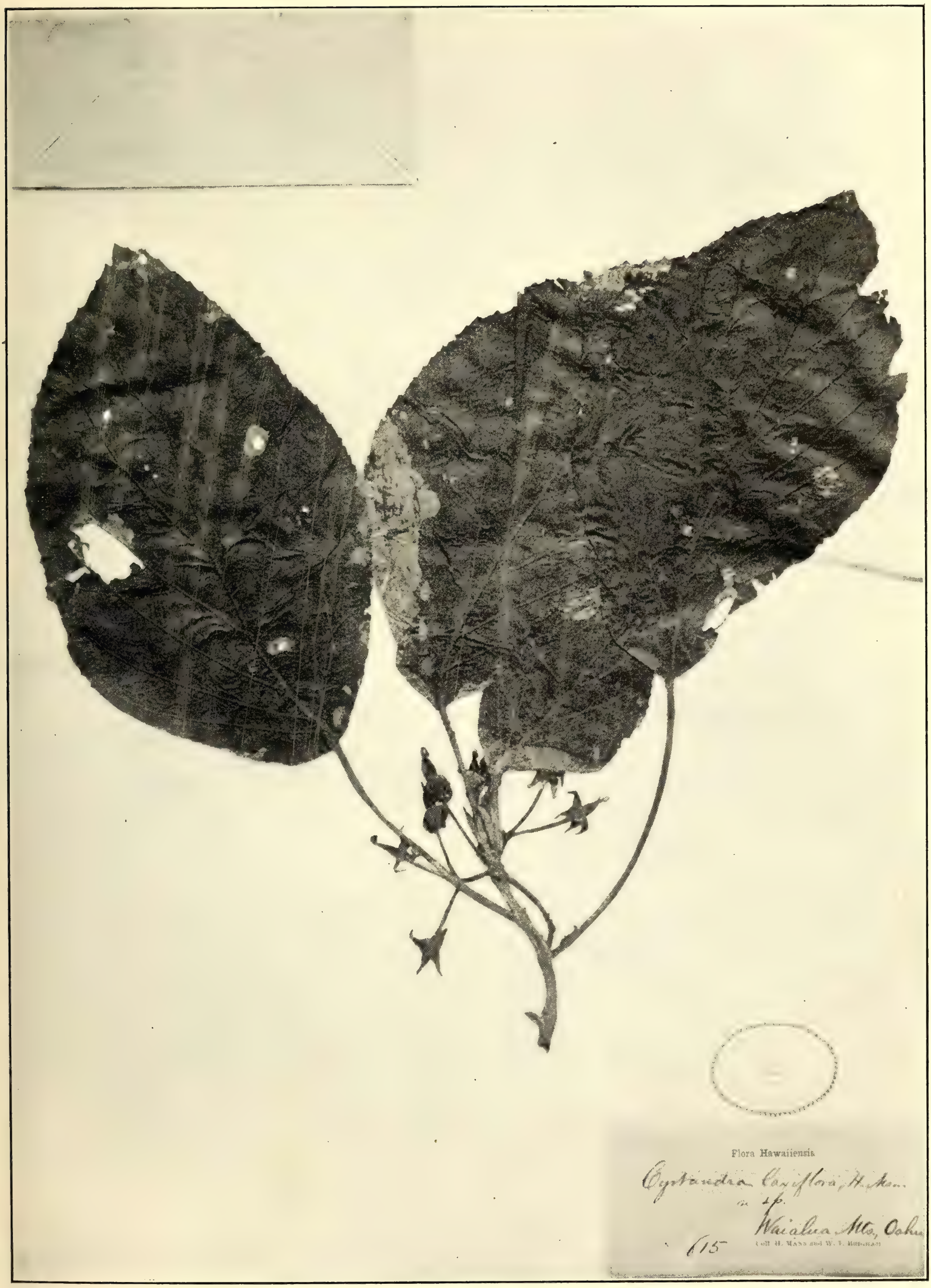
MAUI: Northern slopes of Mt. Haleakala in dense rain forest along the Waikanioi stream at an elevation of 4000 feet, in company with *C. caulescens*, *C. Lydgatei*, *Hillebrandia sanduicensis*, *Gunnera petaloidea*, etc., flowering September 3, 1918, Rock and Hashimoto no. 13117 (type) in herb. College of Hawaii.

C. Hashimotoi, named for my friend and companion T. Hashimoto, who discovered the species, is related to *C. Lydgatei*, from which it differs in the single flowers, the thick, leathery, small leaves, ebracteate peduncle, and the corolla, which is nearly twice as long as the calyx. It is intermediate between the sections Schizocalyces and Chaetocalyces, but must be referred to the former on account of the calycine lobes, which are not green and thin, the single flowers, and the thick, leathery leaves.

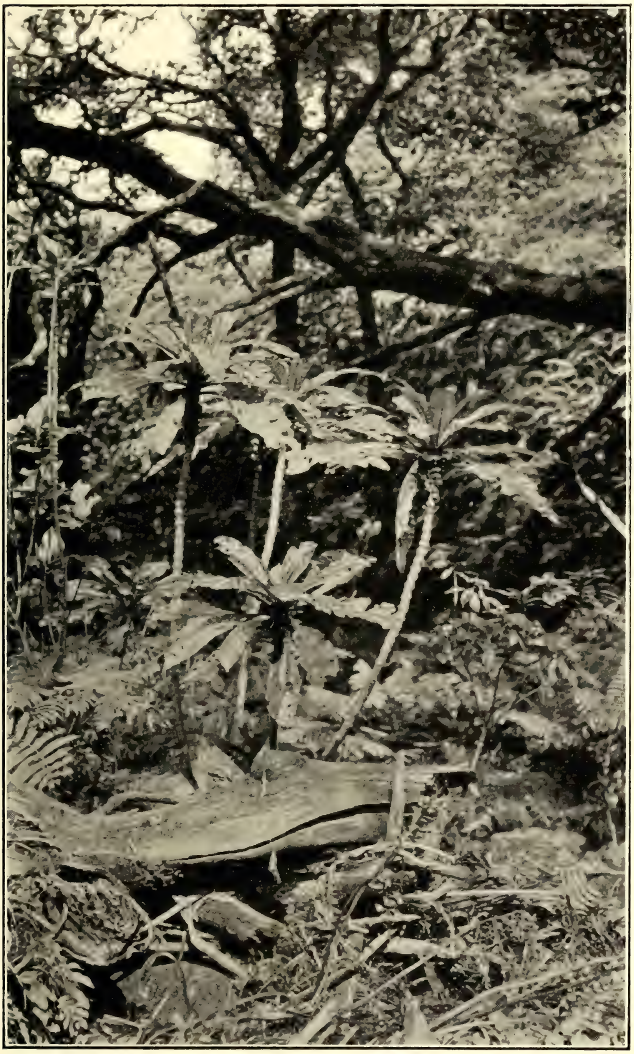
CORRIGENDA

In Rock: Revision of the Hawaiian species of the genus Cyrtandra. Section Cylindrocalyces Hillebr. Amer. Journ. Bot. **4**: 604–623. 1917, on page 613, sixth line, read: "*herb.* (*Wien*) *Vienna*" instead of "herb. Berlin."

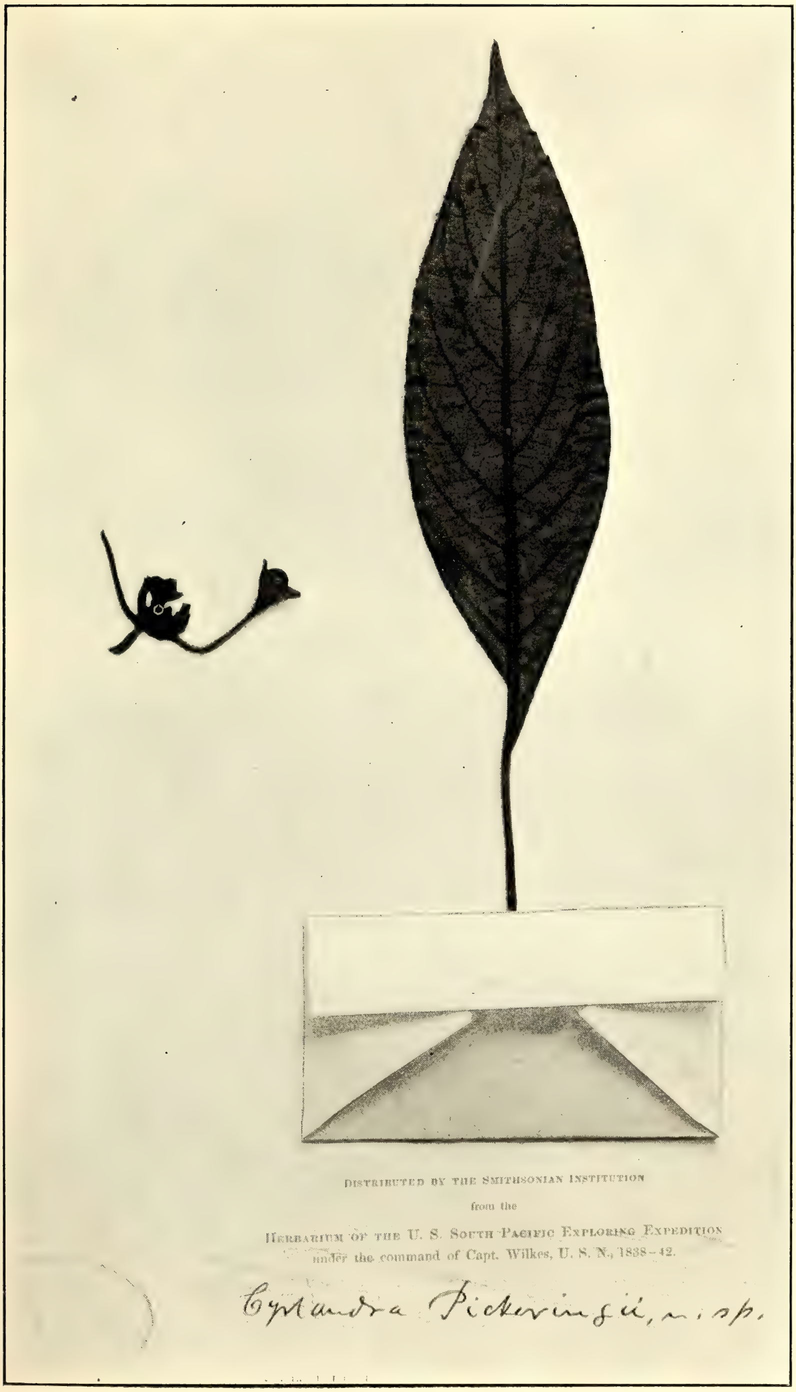
College of Hawaii, Honolulu


Flora Hawaiiensis
Cyrtandra laxiflora, H. Mann
n. sp.
Waialua Mts, Oahu
615
Coll. H. Mann and W. T. Brigham

ROCK: CYRTANDRA LAXIFLORA MANN, EX COLL. MANN AND BRIGHAM IN CORNELL HERBARIUM.



ROCK: CYRTANDRA LIMOSIFOLIA ROCK, GROWING IN THE STREAM BED OF MAPULEHU VALLEY, MOLOKAI.



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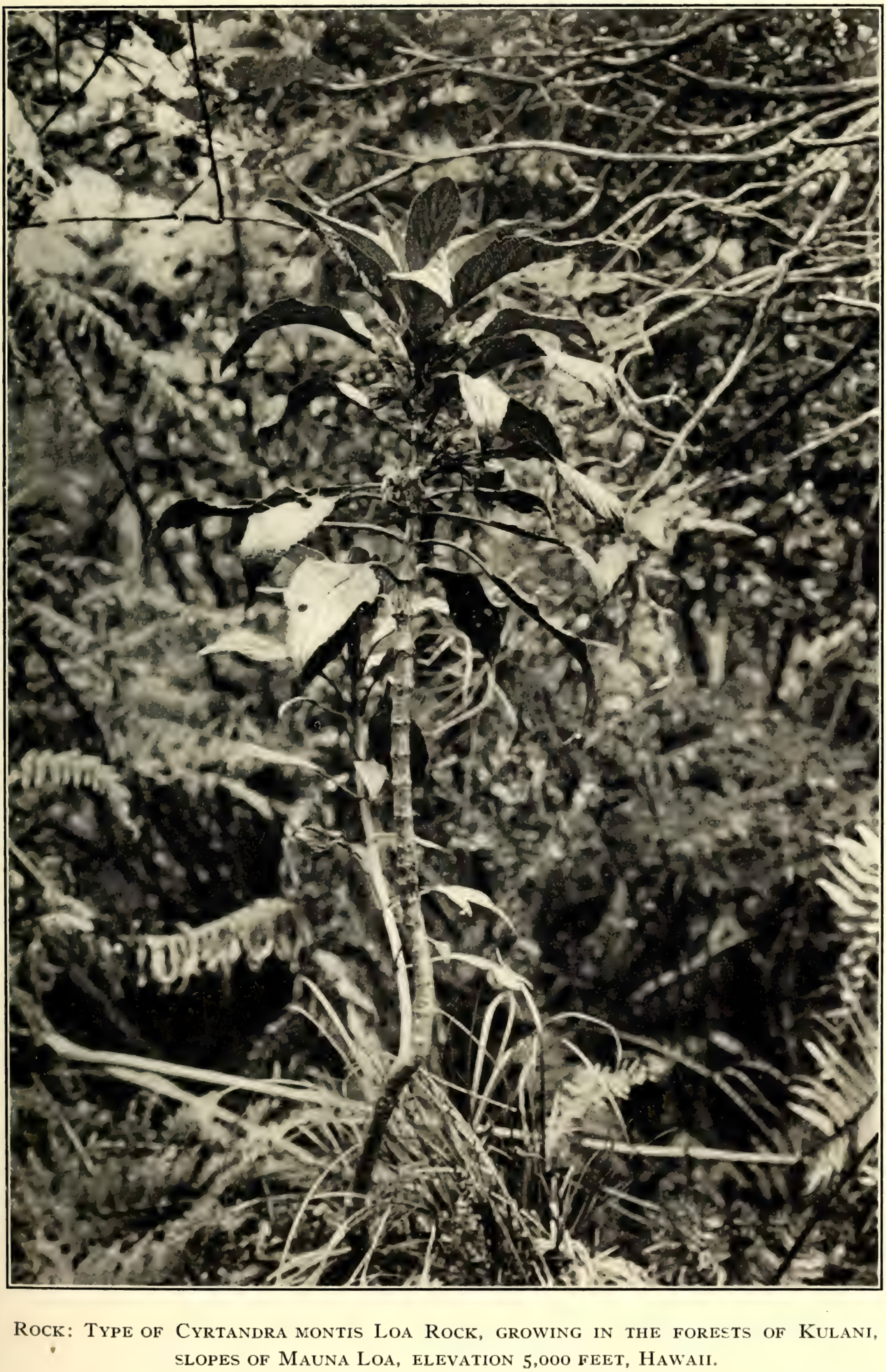
Cyrtandra Pickeringii, n. sp.

ROCK: TYPE OF CYRTANDRA PICKERINGII A. GRAY IN THE GRAY HERBARIUM



ROCK: TYPE OF CYRTANDRA MONTIS LOA ROCK, GROWING IN THE FORESTS OF KULANI, SLOPES OF MAUNA LOA, ELEVATION 5,000 FEET, HAWAII.

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Vol. VI. JUNE, 1919 No. 6

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

Smithsonian Institution
JUL 24 1919
National Museum

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PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
At 41 North Queen Street, Lancaster, Pa.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879

AMERICAN JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

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BOTANICAL SOCIETY OF AMERICA

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Smithsonian Institution
JUL 2 1919
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AMERICAN
JOURNAL OF BOTANY

VOL. VI JUNE, 1919 NO. 6

A STUDY OF PLASTIDS AND MITOCHONDRIA IN PRESSIA AND CORN

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In this paper certain observations upon plastids and mitochondria are recorded. In my opinion it would be premature as yet to formulate any conclusions as to the fundamental significance of these bodies, though the preparations I have obtained are clean-cut and definite.

In order to make clear the position assumed in regard to the structures in question, it may be well to state, at the outset, that the term plastid will be used to include not only the leuco-, chloro-, and chromoplasts, but also the *Anlagen* for the same. The name mitochondria, on the other hand, will be restricted to those granules which are not, in general, preserved by the usual methods of fixation—those which, in other words, are dissolved in acetic acid or in alcohol and are fixed by the use of osmic acid, formalin, etc. The mitochondria, moreover, color more or less specifically with various stains. I shall in general use Benda's term *mitochondria*, rather than others that have been proposed, though the etymology of the word implies a thread-like form not always present.

In cells prepared by what are known as the mitochondrial methods, these bodies, by reason of their number and intense affinity for the dyes become in many cases quite the most striking features of the protoplast. The only reason that they were neglected by cytologists for so long a time is the fact that they are dissolved by the processes commonly used to demonstrate nuclear phenomena.

Interest in the granular constituents of the cytoplasm has greatly increased in the last few years, though the idea of their importance is not a new one. To Altmann, in 1886, is due the formulation of what is generally known as the granular theory of protoplasmic structure. Hanstein, in 1882, had maintained that protoplasm is made up of minute granules, which he termed microsomes, and a homogeneous fluid in which the microsomes float. Altmann, using a special technique, consisting essentially of fixation with osmic acid and potassium dichromate, was able to demonstrate the presence in various tissue cells, as well as in the chromosomes, of numerous granules to which he gave the name of *bioblasts*. These bodies he regarded as possessing an independent existence, and to them he imputed the power of growth and of multiplication by division. He also believed that they

[The **Journal** for May (6: 181–216) was issued June 20, 1919.]

are transformed into the products of secretion such as fats, glycogen, pigments, etc., in the cells. But Altmann, besides claiming for his bioplasts the powers already noted, which are to a certain extent the same as those believed by modern exponents of the mitochondrial theory to reside in the mitochondria, held that the bioplasts are the morphological units of living matter, constituting the essential elements of protoplasm. The difference, in short, between Altmann's hypothesis and the more modern theories of the mitochondria lies in the fact that the bioplasts were postulated to possess an independence and autonomy, to quote Regaud, which the mitochondria as cell organs are not thought to exhibit.

Benda, in 1898, having devised a more specific and definite method of fixation and staining, may be regarded as the founder of the modern mitochondrial theory. He introduced the terms *mitochondria* and *Chondriomiten*, from μίτος a thread, and χόνδριον a grain. *Chondriosome* and *chondriocont*, later introduced by Meves, have also come into use, the former being used synonymously with mitochondria, and the latter being applied by Meves to homogeneous threads. The collective term *chondriome* is also often employed.

Regaud ('11) suggests the possibility that the mitochondria "fix" and "elaborate" the substances necessary for the functioning of the muscle cells, such as glycogen, and that they also perform a similar function in the case of the gland cells, such as the secretory cells of the kidney and of the salivary glands. He says: "*Les mitochondries sont les organites sur lesquels se fixent les substances destinées au fonctionnement chemique de la cellule; ces organites concentrent les substances fixées, les élaborent et les transforment en produits de sécrétion, auxquels ils servent même de supports, dont ils sont les plastes.*" The mitochondria are "*les agents de l'intussusception élective, c'est-à-dire, de l' introduction dans la cellule des substances amenées par le sang.*"

Dubreuil ('13) believes that the mitochondria are responsible for the production of fat in the cells, through a process of differentiation. According to Dubreuil, a lipoid vesicle is first formed, this process being followed by the development of a fat droplet. The diagrams to illustrate this process, showing the mitochondria becoming vesicular and forming both hollow spheres and "hand-mirror-like" forms, exhibit a remarkable similarity to the series of changes which Guilliermond and others show in their illustrations of the production of plastids by the mitochondria in plant cells.

Van der Stricht ('09) has presented some very suggestive studies upon the genesis of yolk-spheres in the egg. He finds that the mitochondria are, at first, confined to a region around the nucleus from which they migrate outward and are gradually transformed into yolk-spheres.

Lewis and Lewis ('15) have employed a novel method for the study of mitochondria with most interesting and suggestive results. Portions of the living embryo of the chick were segregated under aseptic conditions and cultivated in Locke's solution, in hanging drop cultures. In such cultures the mitochondria may be studied in the living, growing condition, fixed while under observation and thus preserved as permanent preparations. Experimental methods may be employed, by which the effect of different stains and reagents may be observed directly. Such preparations, placed in the electric incubator at a temperature of 39° to 40° C., show a beginning of growth in from 10 to 20 hours. The new growth is attached to the cover glass and is several cell layers in thickness at first, thinning out to a single cell layer around the edges. Although conditions are necessarily somewhat different from those of the normal somatic environment, especially as regards circulation, and in the limited supply of oxygen since the cultures must be hermetically sealed, the processes of growth and of mitosis seem to go on as usual for a period of about three days. After this the process slows down, growth ceases, and the cells finally die.

Mitochondria, conforming to the usual criteria, were found to be present in all cells studied. Osmic acid vapor proved to be the best fixative, while the vapors of acetic and other acids caused immediate and total disintegration of the mitochondria. The bodies appeared as threads and granules of the most varied shapes and sizes, just as they have so often been described in fixed preparations. They are not, however, constant in form or in size under these conditions, but are constantly changing in appearance. They are described as undergoing division, as fusing to form larger bodies, and as disappearing and reappearing in the cells in a manner not accounted for. During mitosis they are distributed regularly about the cell, so that they are apportioned to the daughter cells in approximately equal numbers. No connection of the mitochondria with the production of fats, such as described by Dubreuil ('13), was noted in these preparations. In conclusion, the authors state: "The mitochondria are extremely variable bodies, which are continually moving and changing shape in the cytoplasm. They appear to arise in the cytoplasm and to be used up by cellular activity. They are, in all probability, bodies connected with the metabolic activity of the cell."

Lewis and Robertson ('16) found that the above described method of tissue culture was well adapted to the study of spermatogenesis in the grasshopper, *Chorthippus curtipennis.* In the young spermatid the mitochondria were in the form of a granular *Nebenkern.* After certain internal changes, the import of which was not clear, the *Nebenkern* was seen to divide into half-spheres. These half-spheres then elongated to form granular sacs, which, as the tail grew out, formed two irregular strands. These irregular strands finally fused to form "two continuous threads of even width, extending from the centrosome body, or middle piece, almost to the end of the tail."

The authors conclude that "it does not seem possible that bodies which have to do only with the metabolic activities of the cell should undergo such an exact behavior as shown, for instance, by the division of the *Nebenkern* into two equal parts and the development of these two sacs of mitochondria into two long threads of mitochondria in the spermatozoon."

There has been a tendency, especially among the earlier observers of mitochondria in plant cells, to ascribe to these bodies a nuclear origin. A perusal of the literature clearly indicates that the work of Goldschmidt ('04) is largely responsible for this, though the inception of the chromidial hypothesis doubtless owes its origin, as Dobell ('09) states, to the work of R. Hertwig, supplemented by that of Schaudinn, dealing with the occurrence of such bodies in the protozoa.

Meves ('04), working with the tapetal cells in the anthers of *Nymphaea alba*, is credited with having made the first observations of mitochondria in plant cells. Meves shows two very clear-cut and beautifully drawn figures, of which his description is as follows: "*Enthält sie lange, unregelmässig gewundene, ziemlich dicke Fäden, welche sich mit Eisenhämatoxylin intensiv schwarz gefärbt haben. Diese Fäden können nicht wohl etwas anderes sein, als die von tierischen Zellen bekannten Chondromiten.*"

It is not so much the question of origin, whether *sui generis* or chromidial, however, that has engaged the attention of later workers upon plant mitochondria, as that of their relation to other structures in the cell and of their universality. Lewitsky and Pensa, working independently, have advanced a contention which promises to furnish material for controversy for some time to come.

Lewitsky ('10) studies the root-tip and stem-tip of *Asparagus officinalis* treated according to the Benda method and stained with both the Benda stain and haematoxylin. He finds mitochondria corresponding to those described in animal cells, both in general appearance and in staining reaction, with no evidence whatever of a nuclear origin. The mitochondria appear short and rod-shaped, in the dermatogen; somewhat larger and with a tendency to swell at the ends, in the second layer; still larger in the third layer, while deeper in the assimilative tissue "dumb-bell" forms are seen, "similar to those well known in division figures of the chloroplasts." Next, still larger bodies are shown which appear as if they have come from the separation of the two halves of the "dumb-bells." These are followed by figures of the young chloroplasts, and finally by the mature bodies. Meanwhile, the earlier forms of the mitochondria, combined with the "division figures" of the intermediate regions, lead him to conclude that the mitochondria are the *Anlagen* of the chloroplasts. Upon fixing some of the same material in alcohol and acetic acid, Lewitsky found that the mitochondria were no longer to be seen in the cells, while the chloroplasts appeared as usual. This is taken as evidence of a chemical as well as a morphological transformation of the mitochondria, in producing the chloroplasts.

Guilliermond ('11, '13, '14) has published a number of papers in which he describes the mitochondria in all sorts of plants, his purpose being, on the whole, not so much to demonstrate the presence of mitochondria therein as to substantiate his theory of their functional rôle. In addition to portraying the same processes of development as those described by his predecessors, Guilliermond seeks to show that the mitochondria of plant cells possess the same "elaborative" functions that have been postulated for animal mitochondria by Regaud and others. In a resumé of the work upon mitochondria, published in "*La Revue génerale de Botanique*" in 1914, he says: "*Ces recherches démontrent surabondamment que les mitochondries sont des plastes, c'est-à-dire des organites qui élaborent les produits de sécrétion. . . . A la suite de ces recherches, la cellule apparait désormais avec un nouvel élément: le chondriome, dont la présence est aussi constante et joue un rôle aussi essentiel que le noyau. . . . La découverte des mitochondries transforme donc la cytologie.*" Mottier ('18) has discussed the literature on the relations of chondriosomes and plastids and it need not be further summarized here.

OBSERVATIONS

Although I was particularly concerned, in my own investigations, with obtaining evidence as to the relationship between mitochondria and plastids, the mitochondrial methods of fixing and staining were tested first upon animal tissue. A number of preparations were made from the testes of the grasshopper, *Caloptenus femurrubrum*. They were fixed with Benda's solution, with Bensley's, and with Flemming's strong solution, and stained in various ways, in order to compare the results obtained with different combinations.

Benda's method of fixing and staining gave by far the best results, the mitochondria being well differentiated and shown in the characteristic changes through which they pass, as described by Lewis and Robertson ('16) in their observations upon the behavior of the mitochondria during spermatogenesis in the grasshopper, *Chorthippus curtipennis*. In my preparations, Bensley's method gave unsatisfactory results in general protoplasmic differentiation, though the mitochondria were well preserved and stained.

Benda's process, according to the formula given below, was therefore used in most of my plant material.

Fixation. I. Benda's Flemming, 8 days.
(1 percent chromic acid, 15 cc.,
2 percent osmic acid, 4 cc.,
3 drops acetic acid.)
II. Wash in water, 1 hr.
III. Pyroligneous acid (rectified) and chromic acid 1 percent, equal parts, 24 hrs.
IV. Bichromate of potassium, 2 percent, 24 hrs.
V. Wash in water, 24 hrs.
VI. Dehydrate and imbed. Cut 5 microns thick.

Staining. VII. Iron alum, 4 percent, 24 hrs.
VIII. Rinse in distilled water.
IX. Alizarine sodium sulphonate, 24 hrs.
(1 to several cc., saturated alcoholic solution, in 80 to 100 cc. distilled water.)
X. Heat in crystal violet, 3 to 5 min. after vapor rises.
(3 percent alcoholic solution crystal violet and aniline water, equal parts.)
XI. Rinse in distilled water.
XII. Destain in 15 percent acetic acid.
XIII. Wash in running water, 5 to 10 min.
XIV. Dry with filter paper. Dip in absolute alcohol.
XV. Bergamot oil, followed by xylol.

This stain gave a very beautiful result, the mitochondria being colored a dark violet or blue, with a background of old rose. Only in exceptional cases was the background too light to be satisfactory.

Regaud's method of fixing and staining was also employed, to some extent, upon the plant tissues.

Fixation. I. Bichromate of potassium, 3 percent, 80 vols., and commercial formalin, 20 vols., four days.
II. Bichromate of potassium, 3 percent, eight days.
III. Wash in water, 12 hrs.
IV. Dehydrate, imbed, and cut 5 microns thick.
Staining. V. Stain with iron-alum-haematoxylin (Heidenhain's method).

This is the formula as given by Guilliermond, who has used it in much of his work. In my preparations it gave good results, at times, while again the results might be very bad, possibly due to impurities in the formalin The Benda fixation does just as well as a preparation for the iron-alum-haematoxylin as for the crystal violet-alizarin stain, this combination being often used.

CORN

In the root-tips of corn, of the "Canadian Early, Yellow Flint" variety, fixed according to Regaud's formalin-bichromate method, the time being shortened to four hours in the fixative (I), and eight hours in the bichromate (II), the cytoplasm in the embryonic region appears gray and filled with exceedingly numerous jet-black mitochondria, when stained with iron-alum-haematoxylin. In this region the mitochondria are globular, ellipsoid, or short rod-shaped. In the root-cap, next to the tip, they are similar, gradually lengthening from short rod-shaped to elongated, filamentous forms as one passes from the embryonic region toward the periphery of the cap.

In the root-tip proper, passing back into the region of elongated cells in the plerome, a marked change occurs. The mitochondria now appear as elongated, thread-like bodies, seeming to have arisen from the spherical and ovoid forms by a process of lengthening and thinning. Of these elongated forms, many appear hooked, or in some cases vacuolate, at the ends. Mingled with these thread-like structures are others which appear circular, as if a thread had formed a ring, or possibly a globular form had changed in appearance so that it resembles a hollow sphere. At times, chains of small granules are seen, probably due to the breaking up of a filament. Figure 1 shows the large number and greatly varied shapes of the mitochondria in this region.

Passing outward from the region of elongated cells in the plerome and entering the periblem, the cells are found crowded with mitochondria, mostly spherical in shape. It appears as if the mitochondria do not, in general, elongate in this region, though they increase in size, approximately to the same extent in all directions. As will be shown later, these enlarged bodies of the periblem are in reality not mitochondria, but plastids, though they stain in exactly the same manner with the haematoxylin. Whether there are any plastids present in the plerome region as well, I am not as yet prepared to say. Figure 2 shows a cell from the periblem in mitosis, drawn to the same scale as figure 1, namely, nine hundred diameters. Figure 3 shows a similar cell enlarged to twice the size.

As may be seen from these drawings, the mitotic figure is shown very much as it appears in the usual method of fixation, with the exceptions to be noted. The spindle fibers are but faintly shown, if visible at all, although the general outline of the spindle appears as it normally does. The chromosomes are rather attenuated, though they sometimes show a shadowy outline of surrounding material, as if only the central part of their structure had been stained. The difference between this method of fixation and those methods designed primarily for showing nuclear structure, is much more marked in the resting cell. Here the nucleus appears, in general, more uniformly granular and less reticulate than it does in preparations fixed, say, in Flemming's strong fluid. The nucleole also is different, appearing much larger than we are accustomed to see it in fixed material. In short, the mitochondrial methods of fixation do not seem to alter the appearance of the protoplast so much as do the usual types of fixation, since with the mitchondrial methods the structure appears very much as it is described by Lewis and Lewis ('15) and by Lewis and Robertson ('16), in their observations upon living tissue cells.

The root-tips from which the above described preparations were made were grown in the laboratory during the coldest part of the winter and not under constant temperature conditions. To this I attribute the difference between the granular content of the cytoplasm in this material and in the preparations next to be described.

Root-tips of the same variety of corn, grown later in the season and under more favorable conditions of temperature and light, prepared and stained by the Benda method, show a much greater proportion of the enlarged vesicular bodies which stain like mitochondria. In these preparations, however, there is very good evidence that these bodies are in reality plastids, since they show a lighter colored internal portion, evidently consisting of starch. In regions apart from the meristem these bodies are very abundant. In the plerome (fig. 4) the filamentous mitochondria often appear swollen at the ends or sometimes in other portions, while the plastids, ovoid or spherical in shape, may contain from one to several starch grains each as shown in figure 5.

In the periblem, on the other hand, there are in the intermediate regions of the tip, ovoid, spherical, or irregular masses of a much more solid appearance, in general, but often showing a number of discrete spherical granules in their interior. Numerous smaller spherical or ovoid bodies are also present, scattered about through the cytoplasm, which are dark blue in color, taking the stain exactly as do the plastids and the filamentous mitochondria.

These preparations, stained and fixed according to the Benda method, as previously stated, show the nucleus finely granular in appearance and of an old-rose color, as dark, relatively, as shown in the drawings, figures 4 and 5. The nucleole is apparently of a denser consistency and stains a darker shade of the same color. The cytoplasm is very well preserved and stains somewhat lighter than the nuclear material.

A slide of the above described material was freed from paraffin with xylol, bleached for a few minutes in hydrogen peroxide, and treated with potassium iodide-iodine solution, with the object of testing for starch. Under this treatment the bodies which have been referred to as plastids show a bluish color in their interior, but hardly pronounced enough to be considered a convincing demonstration of the presence of starch. A subsequent treatment of the slide with a solution of iodine in chloral hydrate, however, gave better results, differentiating the bodies so that they appear as plastids with included starch grains, as indicated by the blue color of the interior.

Next, a similar slide from the Benda fixation was stained with the Flemming three-color process, the red being left decidedly strong. The mitochondria, both granular and filamentous, are now strongly stained by the safranin, while the plastids are colored blue. In the intermediate regions of the tip, in the periblem area, the plastids are rather lightly stained, and within each of them there are a varying number of spherical granules which are stained by the safranin in the same manner as the mitochondria, except that they are, generally, a brighter red. In figure 6, *a*, *b*, *c*, *d*, and *e*, a number of these plastids are shown with their included red-staining granules. As one leaves the intermediate regions of the tip and proceeds toward the proximal portion, the red bodies within the plastids gradually lose their sharply defined appearance and bright color, becoming gradually dimmer. Still farther back in the tip the red color disappears entirely, the plastids appearing more or less opaque or more uniformly blue in color, as shown in figure 6, *g*, *h*, and *i*.

It has not been possible to determine, so far, how this association of the bright red granules with the light blue plastids comes about, though it might be imagined, from observations I have made, such as the appearance of two darkly staining red granules at the ends of a light blue ellipsoid, that the mitochondria are surrounded by, or become surrounded by, a substance which makes up the body of the plastid; that they divide within this plastid substance and afterwards produce the starch grains within it, or themselves become changed into starch. This appearance suggests a relation to starch formation similar to that of the pyrenoid, as described by McAllister ('14).

In other cases, however, bodies were observed which are made up of a dark red peripheral layer surrounding a light blue center. Both the latter structures and the ellipsoids occur in the intermediate regions of the periblem, between the red-staining mitochondria and the blue plastids with their red, granular inclusions. I wish to emphasize the fact that both the mitochondria and the plastids are exceedingly numerous in the regions indicated and that the staining reactions and the differentiation of the bodies described as occurring in the plastids are very definite. In many cells of the periblem, in the intermediate region of the tip, the nucleus is practically surrounded by a number of large plastids containing red-staining granules, while the cells nearer the tip are crowded with mitochondria which also color strongly with the safranin. Nevertheless, while the existence of these bodies is clearly demonstrable, I do not wish to imply that the seriation to prove their inter-relations is equally evident.

PREISSIA COMMUTATA

The situation in connection with the cytoplasmic inclusions of the liverworts and of the Bryophyta in general, appears to be in special need of investigation, not only on account of the fact that a perusal of the literature shows a considerable difference of opinion as to the real nature of the various bodies in question, but also on account of the very great interest attached to the group by virtue of its intermediate position in relation to the flowering plants on the one hand, and to the algae on the other.

While the interest centers mainly in the plastids and their genetic relations, the oil bodies of the liverworts have received considerable attention from investigators, with no very definite results as far as their real nature and origin is concerned. Pfeffer ('74) is credited with having made the first really fundamental and comprehensive study of the oil bodies. In his opinion they are formed by the aggregation of a large number of small oil droplets which are already visible in the very young cells. While he, at first, maintained that the bodies originate in the cell sap, he later agreed that it might be possible that they come from the cytoplasm, but that they finally lie in the vacuole. He believed the principal constituent of the bodies to be a fatty oil, since their contents dissolve in alcohol, benzol, ether, etc. In addition to the fatty oil, some other material was found to be present, appearing as a residue after the solution of the oil. The membrane which he observed surrounding the bodies after they had been stained with iodine and with cochineal, was apparently composed of some protein material, insoluble in dilute acid and in alkalies. Since the bodies were unchanged after a three-months' cultivation of the plants in darkness, and since they were still present, in such cases, in the very young cells, just as in the plants which had grown in the light, he concluded that they have no significance in nutrition and that they are merely products of excretion.

Wakker ('88) included the oil bodies of the liverworts under the "elaioplasts," as he had named the oil-producing bodies which he had demonstrated in many phanerogams. Although these bodies appear, in life, to lie in the cell sap, Wakker showed by abnormal plasmolyosis that they, in reality, lie in the cytoplasm. He believed them analogous to leucoplasts and chloroplasts, holding that they multiply by division and are distributed to the daughter cells in mitosis.

Von Küster ('94) believes that the oil bodies are formed from a protein "stroma" and that the apparent membrane seen in fixed material is an artefact. Since he was not able to see the membrane in living material, even with the strongest magnification, he considered it a precipitation membrane, formed by the interaction of the oil and the substance of the stroma. He showed that the membranes were not visible in material which had been fixed in osmic acid and stained with gentian violet. He also showed that a double membrane could be formed by the use of dilute alcohol, followed by strong. In regard to the nature of the bodies, he believed with Pfeffer that they are excretion products. He did not believe that the oil bodies undergo division and are handed down from cell to cell, but thought that they are newly formed in each cell.

Garjeanne ('03) believes that it is possible to show that the oil bodies are in reality merely vacuoles filled with oil which is secreted from their walls; that they lie in a half-fluid transition substance; that they increase by division, and that the membrane is an artefact. He admits, however, that the picric acid which he used in his demonstrations acts very rapidly, so that observations upon young cells must be made within one minute after the application of the acid, before disorganization of the cell contents sets in. He compares the oil bodies to the leucoplasts in their origin from *Anlagen*, which he believes to be vacuoles in the case of the leucoplasts also. After being fully formed, the oil bodies, he says, are no longer capable of division, remaining thereafter unchanged. In addition to the vacuoles, or *Anlagen*, of the oil bodies, he describes other minute structures which are similar to the young stages of the oil bodies but which differ from them in their chemical properties. Since, in addition to the *Anlagen* of the oil bodies and to these structures which are similar to them, Garjeanne mentions also the *Anlagen* of the chloroplasts, it would seem that he believes that there are present in the cells of some liverworts, at least three varieties of specifically different granules.

Rivett ('18), in an account of certain observations upon *Alicularia scalaris,* finds that the results of staining or fixing the entire leaves with 2 percent osmic acid "confirm the view that the oil is secreted in vacuoles." This author also finds certain refractive granules present in the cells of both the growing point and the older leaves, which differ in their chemical reactions from the young oil bodies, apparently agreeing with the observations of Garjeanne in this respect. In the meristematic regions a "chondriome-like structure" was observed, but no evidence was found "that the chondriosomes were either transformed directly into plastids by a secretion within their own substance, or that they are the instigators of secretory action on the part of the protoplasm." No evidence was found that the refractive granules were chondriosomes, "since their appearance in the stained mature cells is quite different from that of the chondriome of the actively dividing cells."

As already noted, mitochondria have also been described in the liverworts by Scherrer ('13) and by Mottier, ('18), neither of whom was able to find any connection between these bodies and the chloroplasts. Scherrer made a special study of Anthoceros, a form which possesses the greatest interest since it has a "pyrenoid' in some of its chloroplasts, suggesting a close relationship with the algae in respect to its method of starch formation.

The pyrenoid of Anthoceros has been described by McAllister ('14), who finds that it consists of from 20 to 300 minute lenticular bodies, which lie near the center of the chloroplast and which stain bright red with safranin. McAllister states that there can be no doubt that these bodies are transformed directly into starch, since "there is a gradual change of the color reaction, from the brilliant red of the pyrenoid bodies to the blue of the starch grains." On the other hand, he says that, in the cells of the archesporium, in the spore mother cells, and in the assimilative cells of the sporophyte, starch is formed without the intermediary action of a pyrenoid—apparently arising *de novo* in the chloroplasts.

The observations of Davis ('99) agree with those of McAllister in this respect, since be states that the first clear indication of the chloroplast in the spore mother cells of Anthoceros is the sharp staining of the starch grains—purple with the gentian violet.

McAllister states, further, that there is no doubt that if the *Anlagen* of these bodies (the starch grains of the spore mother cells) are present in the plastids of the archesporial cells, they are too minute to be distinguished with the highest magnifications. This, however, does not necessarily follow, since neither McAllister nor Davis reports having tried the mitochondrial methods of fixation.

Although Anthoceros, in general, has but one chloroplast in each cell, Campbell ('06) has described a species from Jamaica which has several chloroplasts—as many as eight in the cells of the inner tissue—so that the connection of Anthoceros with other liverworts, in this respect, is not so remote as might at first appear. All in all, Anthoceros is obviously a most interesting form and one upon which considerably more work is necessary.

Observations

Portions of the thallus of *Preissia commutata*, upon which the gamete-bearing discs were beginning to appear, were fixed according to Benda's formula, imbedded, and cut 5 microns in thickness. The smallest disc studied was about one millimeter in diameter. Figure 7 represents a portion of such a disc, as it appeared when stained with the Benda method, and with a magnification of three hundred eighty-four diameters. The darker cells in this section, three of which are shown in figure 7, present the same relatively dark appearance in the unstained, unbleached preparations. They are filled with a dense mass of thin-walled, spherical bodies which stain darkly with osmic acid as well as with the mitochondrial stains. Treatment with a preparation of alcannin shows the periphery of these cells made up of an alveolar substance, staining purplish gray, while the central portion contains a mass of material which stains a dark red. These central masses are the "oil bodies" of Pfeffer and others, or the "elaioplasts" of Wakker.

In this section there are also differentiated two other sorts of bodies. The smaller, more uniform variety, which may be seen occupying the periphery of the cells, is apparently of a fatty nature, since they are somewhat darkened in the unbleached cells. They appear granular and plastic, being flattened more or less along the cell walls. There appears to be no difference between the periphery and the interior of these bodies, since no bounding membrane nor any lighter-colored area in the interior can be made out in the stained slides. From the larger ones, two microns or more in diameter, of which there are usually a larger number of about the same dimensions in these cells, they seem to grade down to extremely minute granules.

The other bodies vary much more in size, there being no two of any one size in the cell. They include, doubtless, the bodies described as oil droplets by Pfeffer. Figures 8, 9, and 10 show a few of the cells taken from the same group as figure 7, but more highly magnified. The more uniformly colored bodies in these cells, mostly seen in side view around the periphery of the cell, belong to the first class, while the more rounded ones, with dark borders, belong to the second class. Of the latter, the larger ones may appear to be in a state of division or fusion, a number, of varying sizes, often appearing in groups; figure 11, plate II, shows a peripheral cell from a young disc containing the two sorts of bodies of the second class.

Returning to figure 7, as one proceeds from the periphery of the young disc toward its center, the bodies of the second class appear, on the whole, smaller. There seems to be a gradual decrease in their size correlated with an increase in their number, up to a depth of about two cell layers below the areolae. Here, bodies of this class begin to decrease in number, indicating a diminution in the amount of oil in the cells, while bodies containing from one to several starch grains begin to be seen: figures 8 and 9.

This seriation, suggesting that at least some of the bodies are plastids, is better shown in figures 13 and 14, taken together, which were drawn from a somewhat older disc than that from which figure 7 was taken. Figure 14 shows two cells from still deeper within the disc, as compared with figure 13, from a central lenticular area in which all the cells are of this type and packed with storage starch. These plastids, for such they appear to be, are stuffed with starch and now appear merely as enveloping films, enclosing and separating the starch grains. The character of these starch grains, which show a definite hilum when stained in certain ways, as well as their general appearance and distribution, would indicate that they are, as already suggested, storage starch and that the grains are very likely stratified. It was also found on staining such a section as that shown in figure 12 with iodine, that the starch reaction was given by all the bodies of this character, even to some of the smaller ones in the peripheral layer of the disc. Figure 15 represents a series of stages in outline as they are seen in the development of starch in this disc.

In the chloroplasts of the thallus, starch is also present in large quantities, and toward the interior of the thallus there is a region in which the cells are moderately full of swollen plastids, each containing a number of plump starch grains, not at all lenticular in appearance as they are so often figured. Lenticular-shaped starch grains are found, however, in the chloroplasts of cells at and near the periphery, especially in the vicinity of the growing point of the thallus. In the cells of the disc which contain starch, as well as in those of the thallus, the smaller, more plastic, and darker-staining bodies are still seen, arranged around the periphery. The largest of these should be the young chloroplasts.

In the apical cell of the thallus and in its immediate vicinity, bodies may be seen which I take to be the same as those already described from the peripheral cells of the young disc, though they are much smaller in size. In this region, also, and especially in the filamentous growths therefrom, mitochondria of various shapes appear, very much as described by Mottier ('18) for Marchantia.

Discussion

It was at one time the accepted belief among botanists that chloroplasts arise *de novo* in the cytoplasm. This is definitely stated to be the case by

Sachs, in his Text-book of Botany, in 1882, where the process is compared to that of so-called free-cell formation. Since the work of Schimper, afterwards confirmed by that of A. Meyer, the opinion has become general that the three kinds of plastids found in plant cells, namely, leuco-, chloro-, and chromoplasts, are derived from minute, undifferentiated plastids which are *sui generis* structures of the cytoplasm. These chromatophores, as they are often called, were described, when seen in the living cell, as small, colorless, highly refractive bodies, recognizable in the egg and also in the embryonic cells. In older cells they have been said to retain the same appearance in some cases, while in others they become differentiated into leuco-, chloro-, and chromoplasts.

Schimper and Meyer believed that the undifferentiated plastids multiply by division and are handed down from generation to generation—that they have an individual existence in the cells. Considerable difficulty, however, was encountered by them in their attempts to demonstrate the presence of the plastids in the egg, owing to the fact that they were not easily seen in the living cells, and, as was admitted, they were difficult to stain at that stage. As Guilliermond expresses it, "that part of their theory remained very hypothetical."

When the mitochondria were demonstrated, by means of a special technique, their study was first taken up by the zoologists, as has been shown, and special functions in the cell metabolism were imputed to them by Meves and others. Pensa is credited with having made the first observations tending to show that the mitochondria of plants may, possibly, be transformed plastids. This idea, developed by Lewitsky and Pensa and supported by numerous observations which have already been noted, was taken up by Guilliermond, who has attempted particularly to harmonize the functions of the mitochondria of plant cells with the theories concerning those of the mitochondria of animal cells as postulated by Meves, Duesberg, Regaud, Dubreuil, and others. He has confirmed the observations of Lewitsky and of Pensa by work upon a number of plants, including the seedling of barley. Here the mitochondria, followed from the meristem toward the green tip of the plumule, are shown as filamentous at first, followed by shorter and thicker forms which are sometimes dumb-bell shaped. From the appearance in succeeding cells of bodies which have the appearance of the separated halves of the "dumb-bells," he believes that the latter divide. These bodies are followed by more rounded forms with a light center and a darker border. Finally, in the tip of the plumule, the mature chloroplasts are seen. While this series is considered by Guilliermond a very convincing proof of the mitochondrial origin of the chloroplasts, it is open to the objection that there seems to be no way of demonstrating that the *Anlagen* of the plastids are actually mitochondria and not merely young plastids.

On the other hand, the attempts of Rudolph, Sapêhin, Mottier, and others to substantiate their contention that the *Anlagen* of the plastids are different from the mitochondria fail for the same reason—they are unable to demonstrate such a difference. Guilliermond ('14) claims that he is, in reality, upholding the Schimper-Meyer theory in that he is bridging a gap which the latter, with their cruder technique, were unable to fill. Schmidt ('12) also maintains that the work of Guilliermond and others confirms the Schimper-Meyer theory, but for a very different reason: they have simply been demonstrating the earlier stages of the plastids, according to Schmidt.

Lewitsky, however, claims to have shown that there are no other *Anlagen* of the chloroplasts than the mitochondria. His findings, with respect to *Asparagus officinalis*, which have already been referred to, are specifically as follows: a stem-tip of *Asparagus officinalis* was fixed with alcohol, 3 parts, and acetic acid, 1 part. This was stained with iron-alum-haematoxylin and light green. In the third and fourth cell-layers from above, where, in the preparations fixed by the Benda method, the somewhat large "chromatophore-dumb-bells" were found, these were no longer to be seen; only the usual "*Plasmagerüst*" was present. Since the mitochondria are destroyed by acetic acid and alcohol, and since all of the *Anlagen*, including the dumb-bell forms, disappear with the mitochondria under fixation with a combination of the above named reagents, these facts are taken as conclusive evidence of the identity of the mitochondria with the *Anlagen* of the plastids.

Conclusions

My own observations may be briefly summarized as follows:

1. As to size, an unbroken series of bodies, from mitochondria to plastids, can be traced in the root-tip cells of *Zea Mays* from the embryonic region backward. In Preissia this seriation is not so obvious.

2. The contention that such definitely staining bodies as the mitochondria exist and are normal constituents of the cytoplasm can hardly be questioned.

3. The evidence for the division of the mitochondria as well as that for their functions in heredity seems to me to be inadequate.

4. The further fundamental question as to the relation of the mitochondria to the remainder of the cytoplasm and the nature of the material in which they are imbedded, has not been cleared up.

5. Red-staining bodies are present in the plastids of corn, and, in some cases, in those of Preissia also.

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EXPLANATION OF PLATES XXXIII–XXXIV

All figures were drawn with the aid of the camera lucida, with a Leitz No. 3 ocular and Leitz 1/16 in. oil immersion lens, with the following exceptions: In figure 7 a Leitz No. 6 objective was used; in figures 13 and 14, a Spencer 1/12 in. oil immersion lens.

PLATE XXXIII

FIG. 1. Cell from plerome of root-tip of corn, showing mitochondria. × 900.

FIG. 2. Cell from periblem of same preparation, showing mitotic figure and ovoid and spherical mitochondria. × 900.

FIG. 3. Cell from same region showing similar structures. × 1800. All of above from Regaud's fixation and haematoxylin stain.

FIG. 4. Cell from plerome of corn root-tip, from plant grown under more favorable conditions. Benda's method of fixation and staining. Vesicular structures show light blue interior. × 900.

FIG. 5. Two cells from periblem of same preparation. Mitochondria ovoid or spherical. × 900.

FIG. 6. Series of plastids from same preparation, stained with Flemming's tri-color process. First six in series have red-staining granular inclusions; *g*, *h*, and *i* are stained blue. Iodine test shows starch grains in latter. (FIG. 6 on Plate XXXIV.)

FIG. 7. Section from young disc of Preissia, showing distribution of the granular material in the cells and in the thallus as a whole. × 385.

FIGS. 8, 9, and 10. Cells taken from same section, showing plastids, etc., on larger scale. × 900.

Plate XXXIV

Fig. 11. Cell from periphery of young disc of Preissia. Benda's method (unbleached). Large bodies with dark borders, violet; smaller, uniformly-colored structures, brown. × 1800.

Fig. 12. Cell from same region, showing only larger bodies. × 1800.

Fig. 13. Portion of older disc, showing development of plastids, with large grains of storage starch. Iodine stain, with Benda's fixation. × 800.

Fig. 14. Two cells from central part of same disc, showing fully developed grains. × 800.

Fig. 15. Series of developing starch grains, from near the periphery of the disc to its center. × 900. (Fig. 15 on Plate XXXIII.)

Fig. 16. Cell from thallus of Preissia. Benda's method (unbleached). Large "chloro-leucoplasts" light blue, with dark violet peripheries and filled with starch grains. Smaller bodies, dark brown. × 900.

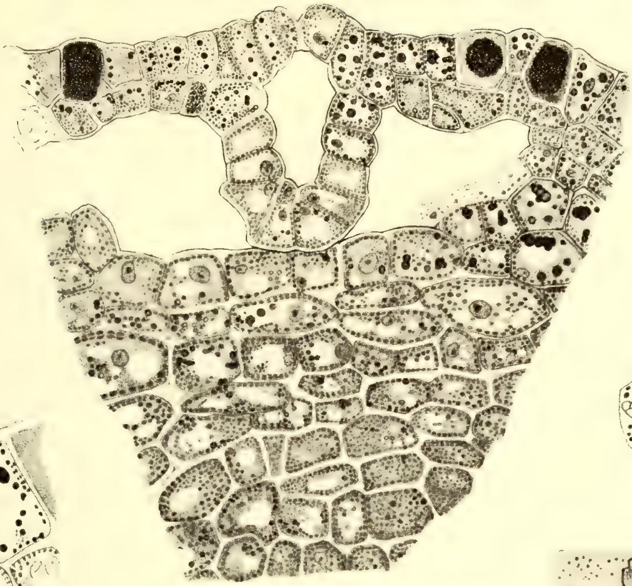


Fig. 7.

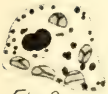
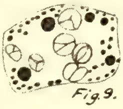


Fig 8.



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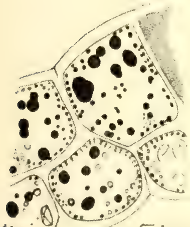

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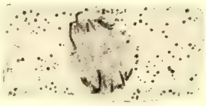
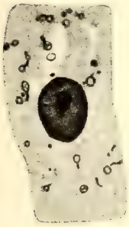


Fig. 2.



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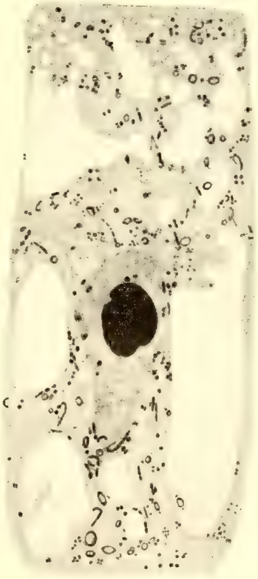

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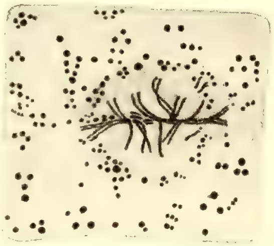

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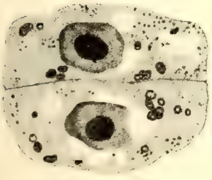

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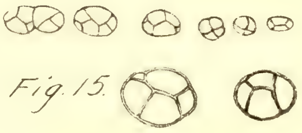
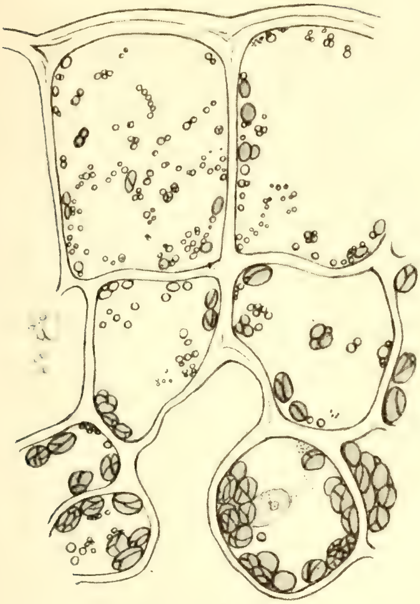
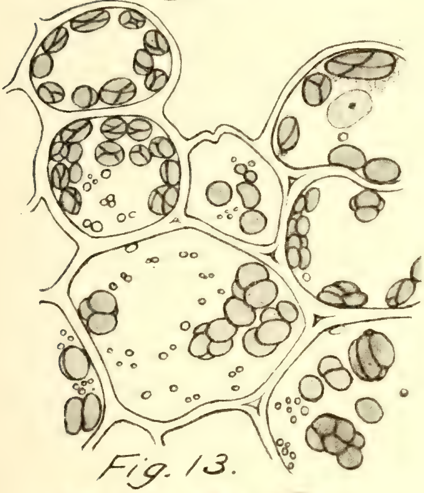


Fig. 15.

TWISS: A study of plastids and mitochondria.



Areola



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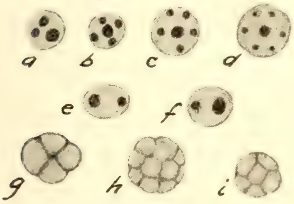

Fig. 6.

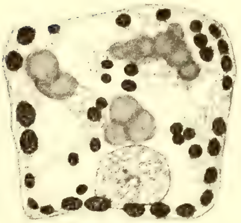

Fig. 11.

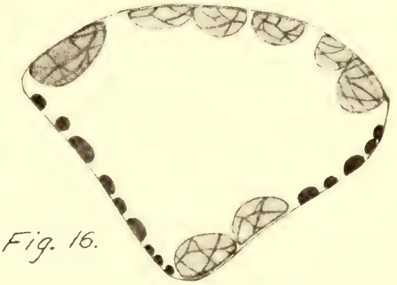

Fig. 16.

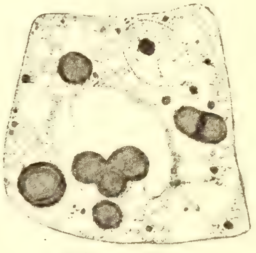

Fig. 12.

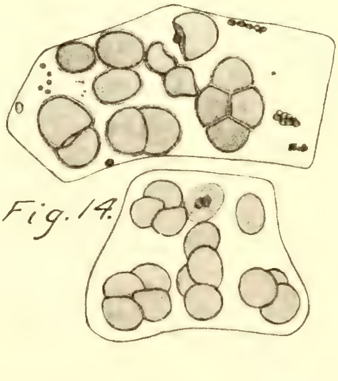

Fig. 14.

Twiss: A study of plastids and mitochondria.

THE EFFECT OF THE ENDROT FUNGUS ON CRANBERRIES

Neil E. Stevens and Fred W. Morse[1]

Introduction

The fungi which cause decay of cranberry (*Oxycoccus macrocarpus*) fruits have been the object of considerable study. Very little, however, has been written as to the chemical or morphological changes produced in the berries by any of these fungi. This is due chiefly to the practical impossibility of obtaining for inoculation cranberries which may safely be assumed to be free from fungi (5, pp. 21–23). The small size of the fruit, and the relatively slow rate at which the fungi grow render it impracticable to quarter the fruit and inoculate two portions, holding the others as controls, as was successfully done by Hawkins in peaches and potatoes (3, 4). Certain characteristics of *Fusicoccum putrefaciens* Shear are, however, so distinctive as to make it possible to secure a quantity of berries affected by this fungus and apparently free from all others.

Distinctive Characters of Growth of Fusicoccum Putrefaciens

According to Shear (6, p. 35) *F. putrefaciens* is of first importance as a cause of rot of cranberries and has been found on different varieties in Maine, Massachusetts, New Jersey, Michigan, Wisconsin, Oregon, and Washington. A very striking characteristic of this fungus in its attack on the cranberry is the fact that, so far as has been observed, decay always begins at the end of the berry. This has given rise to the name "endrot" for this disease.

The importance of endrot in Massachusetts has been pointed out by Franklin (1, p. 100; 2). In temperature studies of cranberry rot fungi it has been found that while the minimum temperature for growth of most of them is above 5° C., the endrot fungus will grow somewhat even at 0° C. (8). Unlike most cranberry fungi, *F. putrefaciens* when grown in pure culture has a bright-colored mycelium, and the fungus is thus readily identified even before it fruits (6, p. 39).

As described by Shear (6, p. 36), "Endrot first appears as a softening of the tissues accompanied by a slightly yellowish or brownish yellow watery discoloration of the skin. The diseased part is lighter-colored than the sound portion of the berry." The rot begins usually (at least in Massachusetts) at the side of the calyx and spreads until the entire inner tissue of the berry is reduced to a pulp, though the epidermis is rarely broken. The fruit thus "becomes soft and elastic to the touch, but remains turgid."

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[1] The chemical work described in this paper was done by Morse, the histological work by Stevens.

METHOD OF SELECTING MATERIAL FOR STUDY

The prevalence of the endrot fungus on the Howes variety in Massachusetts, its relatively late development in the fruit, its characteristic place of attack, and its ability to grow at low temperatures, were utilized in selecting berries which were infected only by this fungus. About half a bushel of Howes, picked during September, 1917, from the state experiment bog at East Wareham, Massachusetts, were placed in storage in a crate with slatted sides and bottom so that abundant aeration was obtained. These berries were allowed to remain in storage about one month at temperatures which ranged from 15° C. to 20° C. Late in October, 1917, these berries were sorted into three lots: (1) those showing a small decayed area close to the calyx which appeared to be typical endrot, (2) perfect berries with no indication of decay, and (3) injured berries or decayed ones which did not show endrot in early stages. Lot 3 was immediately discarded, and lots 1 and 2 returned to storage, the temperature of the storeroom at this time being from 10° C. to 5° C.

On December 19, 1917, the storage lots were re-examined and material was selected for comparative study. This consisted of partly rotten berries from lot 1 (those which in October had shown incipient endrot) in which the rot had developed typically, and of sound normal berries from lot 2. Some of the berries were fixed immediately for microscopic study. Both lots were then stored at a temperature somewhat below 5° C., and portions were removed during January, February, and March. A few berries from each portion were fixed for microscopic study, and the remainder were used for chemical analysis.

HISTOLOGICAL OBSERVATIONS[2]

Fusicoccum putrefaciens is evidently (Fig. 1, *A*) one of the fungi in which the hyphae often grow directly into the host cells, as distinguished from those fungi in which the hyphae grow in the intercellular spaces and either do not enter the host cells as does *Rhizopus nigricans* (7), or develop specialized haustoria which penetrate the cell walls.

While microscopic study furnished no evidence as to the way in which the fungus breaks through the cell walls of its host, there is some indication that the hyphae grow rather more readily between or within the cells than through the walls, since the hyphae frequently grow for considerable distances between the cells without breaking through and often branch at cell intersections (Fig. 1, *B* and *C*).

Moreover, the hyphae are often constricted where they pass through the cell wall (Fig. 2, *A* and *B*), and a few cases were found in which several hyphae entered a cell through a single opening (Fig. 2, *C*). That the hyphae readily grow through the protoplasm and into the vacuole is evident (Fig. 3, *A*), and in this case as in some others the nucleus persists and is readily distinguished after the cytoplasm is largely disorganized.

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[2] The material for microscopic study was fixed in a solution consisting of equal parts of glacial acetic acid and absolute alcohol, imbedded in paraffin, and the sections cut from $7\ \mu$ to $10\ \mu$ thick. Several stains were used, safranin with Delafield's haematoxylin, and safranin with "light green" in clove oil proving especially useful.

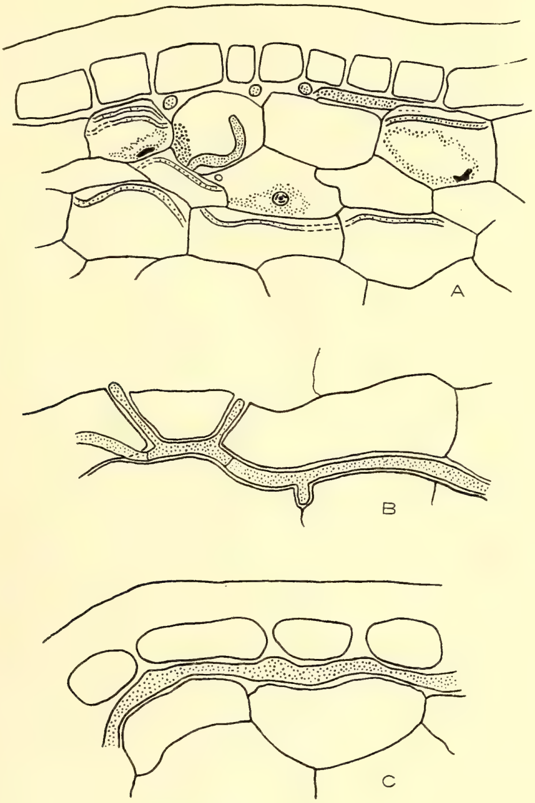

FIG. 1, *A*. Section of cranberry tissue containing hyphae of endrot fungus. Note thick cuticle, small, nearly rectangular epidermal cells, and below these the storage cells of the cranberry. Between and within the storage cells are hyphae of *Fusicoccum putrefaciens*. × 450. *B*. Section showing hyphae of *F. putrefaciens* between cells of cranberry and in three cases branching at cell intersections. × 450. *C*. Section of cranberry tissue showing single hypha between epidermal cells and subjacent storage cells. × 450.

With the apparent exception of the seeds, the fungus grows readily in all parts of the berry. Hyphae are found in the vascular bundles, as well as in the cells lining the seed cavity (Fig. 3, *A*) and in those immediately below the epidermis (Fig. 1, *A* and *C*).

In the slides examined, no case was found in which the mycelium had penetrated the cuticle, although in numerous instances the hyphae grew close to it and even under it for considerable distances. The fruiting bodies (Fig. 3 *B*) apparently develop outside the epidermal cells (the color-bearing cells) under the cuticle, which latter they finally rupture mechanically.

Histological study yielded no direct evidence as to the manner of entrance of the fungus into the berry. In the earliest stages of decay examined, hyphae were abundant in the region close to and beside the calyx. While this may be the region in which the fungus often gains entrance, it is improbable that this is the only place, since in Wisconsin endrot commonly is first apparent at the stem end.

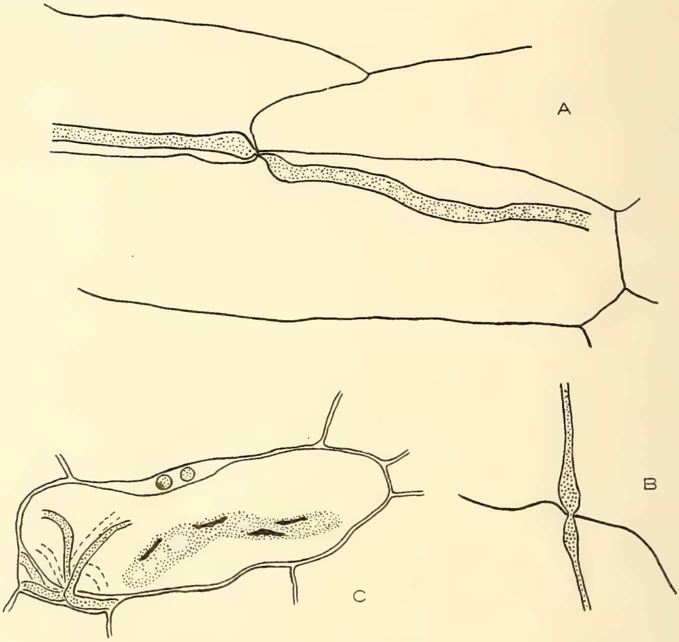

FIG. 2, *A* and *B*. Sections showing constrictions in hyphae of endrot fungus where they pass through walls of cranberry cells. × 700. *C*. Storage cell of cranberry showing several hyphae of endrot fungus entering through a single opening. × 450.

CHEMICAL OBSERVATIONS

During the winter of 1918, on the dates indicated in Table 1, three samples of cranberries of the Howes variety, selected according to the method outlined above because they were infected by the endrot fungus, were received at the Massachusetts Experiment Station, Amherst, Mass. These samples had been held in storage under the conditions described, first at East Wareham, Mass., and later at Washington, D. C. In most of the berries the decay had progressed so far that the berries were very soft. One of the sound samples cited in Table 1 had been stored under the same conditions as the decayed samples.

Soon after a sample was received, duplicate charges of 50 grams each were prepared for the determination of total sugars and total acids. The remainder of the fruit was weighed and dried at a temperature of about

55° C. To promote rapid drying, it was necessary to puncture each berry in several places with a large pin, as the sound epidermis of the cranberry is nearly water-proof. The dried berries were weighed as air-dry, pulverized in a porcelain mortar, and preserved in tight bottles for subsequent analysis.

The charge for sugar and acid was mashed in a porcelain mortar, and the mass was then washed into a 500 cc. volumetric flask with about 300 cc. of water by the aid of a wash bottle and a short-stemmed funnel. The flask was then set on the steam bath for about one hour, and the contents was repeatedly shaken so that the whole mass formed a thick liquid. After cooling the flask and contents to room temperature, the flask was filled to the 500 cc. mark and thoroughly shaken. The whole mass was thrown on a fluted filter large enough to contain it, and the funnel was covered with a watch glass while the filtrate was collected in a flask. The first 20 to 30 cc. of filtrate was thrown away.

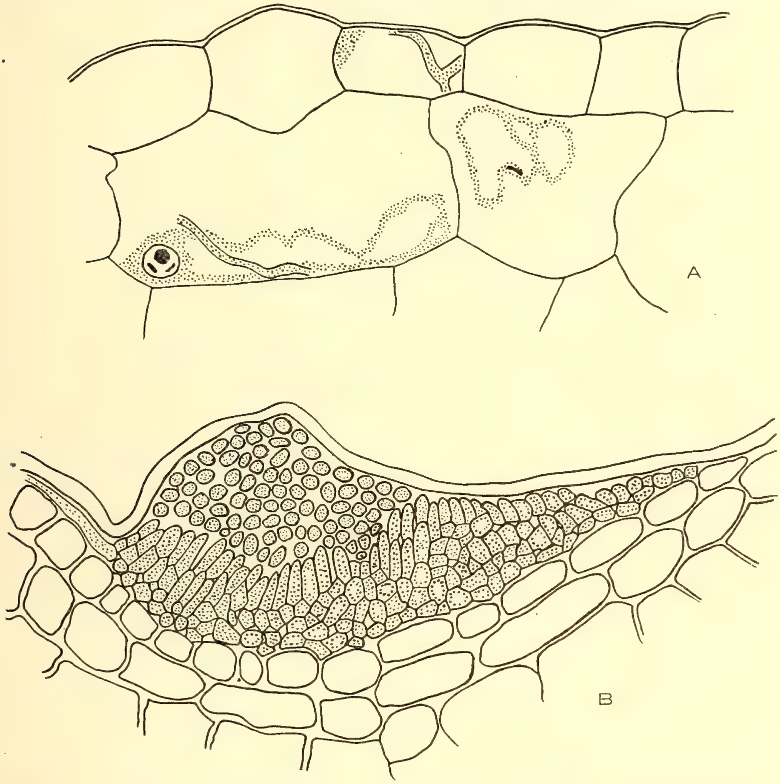

FIG. 3, *A*. Section through portion of storage tissue of cranberry adjoining seed cavity. Note slight thickening of cell walls lining the seed cavity, hyphae of *F. putrefaciens* in one of these cells lining seed cavity, and inside the protoplasm of an adjacent cell. The nucleus in this cell is still readily distinguishable. × 450. *B*. Young pycnidium of *F. putrefaciens* developing just under the cuticle of calyx. × 450.

Aliquots of the filtrate were clarified with dry lead subacetate, the excess of lead was removed by dry sodium carbonate, and the inversion and determination of the total sugars were accomplished in the usual manner.

Aliquots of the filtrate were diluted with water and titrated with standard sodium hydroxide for the total acids. The color of cranberry-juice would be expected to hide the end-point of any indicator; but as the alkali is added to the solution the color gradually changes and fades until it is a pale gray tint. A few more drops of the alkali will then produce a reasonably sharp end with phenolphthalein, which was used in all our work.

The pulverized, air-dry material was used for determinations of dry matter, ash, protein, fiber, and ether extract, which were made by the conventional methods (9).

The analytical results together with results obtained on sound berries are given in table 1.

TABLE 1. *Chemical composition of cranberries (Howes variety)*

Berries affected with endrot compared with sound fruit

Fresh Fruit

	Total Sugars Percent	Total Acid Percent	Dry Matter Percent
Rotten, January 3, 1918	2.91	2.73	11.03
Rotten, February 19, 1918	2.41	2.32	11.82
Rotten, March 5, 1918	2.83	2.25	11.64
Sound, March 5, 1918	3.40	2.21	11.82
Sound, October, 1917	3.97	2.28	12.90

Dry Matter

	Ash Percent	Protein Percent	Fiber Percent	Ether Extract Percent
Rotten, January 3, 1918	1.48	3.45	12.76	6.05
Rotten, February 19, 1918	1.47	3.54	13.90	6.13
Rotten, March 5, 1918	1.40	3.43	11.74	5.44
Sound, March 5, 1918	1.28	3.10	12.21	5.17
Sound, October, 1917	1.28	3.22	12.00	7.62

The total acid was calculated as citric acid, although the cranberry is known to contain benzoic acid, while quantitative tests showed the presence of either tartaric or malic acid or both in addition to citric. The ether extract is not true fat, but contains what is probably a wax or resin from the skin, as well as much of the acids, which are somewhat soluble in ether. The chemical determination of cellulose has not yet reached the precision required for a study like this and methods for the cellulose derivatives are still less suitable, so that only the general determination of crude fiber was attempted.

The only constituent of the fruit sufficiently affected by the rot to be manifested in the chemical analysis, is the total sugar. All the other changes in comparison with the sound fruit are apparently due to concentration as a corollary to the sugar consumption by the fungus.

COMPARISON OF CHEMICAL AND HISTOLOGICAL OBSERVATIONS

The chemical analysis of sound cranberries and of those affected by the endrot fungus shows that the only marked difference is that rotten berries contain considerably less sugar. It is apparent that the endrot fungus uses the sugar contained in the cells of the cranberry; this was to be expected from the fact that the hyphae frequently penetrate the cells and enter the vacuoles. In view of the high acid content of the cranberry and of the fact that *F. putrefaciens* is known only on that fruit, it is interesting to note that there is no considerable change in the total acid content. As the various acids known to occur were not separately determined, the possibility that the fungus uses one or more of the acids and produces some other acid in approximately equal amounts, has not been eliminated. At least one fungus producing decay of fruits has been reported to produce acid, and others have been reported as using acids (see summary in 3, p. 80). Chemical and histological observations agree in indicating that the fungus has little, if any, effect on the cuticle of the berry.

SUMMARY

The endrot fungus has such distinctive characters that it was possible to select cranberries rotted by this fungus alone. A histological study of those selected berries showed that the endrot fungus grows in all parts of the berry except the seeds and the cuticle and is able to penetrate cell walls and protoplasm. A chemical study showed that the sugar content of berries rotted by the endrot fungus is much lower than that of sound fruit. The fungus thus apparently utilizes these sugars.

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MASSACHUSETTS EXPERIMENT STATION, AMHERST.

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4. ——. Effect of certain species of Fusarium on the composition of the potato tuber. Journ. Agr. Res. **6**: 183–196. 1916.
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ENDOTHIA PIGMENTS II

ENDOTHINE RED[1]

Chas. E. Sando

In 1911 Panatanelli[2] noted a yellow pigment occurring in *Endothia parasitica* and attempted to identify the substance as a lipochrome. Later, Anderson[3] claimed that the pigment was closely related to aurin. Shear and Stevens[4] were the first to point out that certain species of Endothia produced a "perilla purple" color when grown on starchy media. In a general study of the pigments produced by species of Endothia, the results of which are presented in the first paper of this series, Hawkins and Stevens[5] showed that three distinct coloring matters are elaborated. Two of these are yellow and have been designated pigments A and C, and the other, which is a brilliant red, has been designated pigment B. With regard to the similarity of any of these pigments to a lipochrome, these authors make the following statement:

"It is obvious then that these pigments are lacking in many of the properties of lipochrome and there is little reason at present for assuming that they belong in this rather indefinite group."

They do state, however, that the pigments from *E. parasitica* bear some similarity to aurin, but not enough to warrant the conclusion that they are the same.

All three pigments described by Hawkins and Stevens are found in *Endothia fluens*, pigment B abundantly. This species therefore afforded an excellent source of material for a further chemical study of the red pigment. This paper deals entirely with the chemistry of the coloring matter designated as pigment B by Hawkins and Stevens, which is probably the source of the "perilla purple" of Shear and Stevens. Since the substance appears to be a new coloring matter, the name *endothine red* is proposed.

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[1] The work here reported was carried out under the general supervision of Dr. Lon A. Hawkins as a part of a cooperation between the Office of Plant Physiological and Fermentation Investigations and the Office of Horticultural and Pomological Investigations, Bureau of Plant Industry. Published by permission of the Secretary of Agriclture.

[2] Panatanelli, E. Sul parassitismo di *Diaporthe parasitica* Murr. per il Castagno. Rendiconti della Accad. Lincei Roma, Classe di Sceinze, Fisiche, Matematiche e Naturali. V. **20**: 366–372. 1911.

[3] Anderson, P. J. The morphology and life history of the chestnut-blight fungus. Bull. Penn. Chestnut Tree Blight Comm. **7**: 1–43. 1913.

[4] Shear, C. L., and Stevens, Neil E. Cultural characters of the chestnut-blight fungus and its near relatives. U. S. Dept. Agr. Bur. Pl. Ind. Circ. **131**: 3–18. 1913.

[5] Hawkins, Lon A., and Stevens, Neil E. Endothia pigments. I. Amer. Journ. Bot. **4**: 336–353. 1917.

EXPERIMENTAL

For the purpose of obtaining a sufficient quantity[1] of pigment for investigation, *Endothia fluens* was grown in flasks on sterilized rice. The culture medium with the mycelium was dried and ground, and the coloring matter obtained in the manner described by Hawkins and Stevens. The method is briefly as follows:

A cold alcoholic extract of the powder was evaporated under vacuum until most of the alcohol had been removed, and the residue, which consisted of a mixture of the impure yellow and red substances, was treated with a large volume of water. This treatment caused a heavy reddish-yellow precipitate to separate, leaving the supernatant liquid dark red. A smaller amount of the red compound was recovered from the mother liquor. The reddish-yellow precipitate after thorough extraction with ether left a residue which consisted mostly of endothine red in a semi-crystalline state. By repeated recrystallization from alcohol it was finally obtained comparatively pure. It was possible, also, to purify the pigment by dissolving it in boiling water and allowing it to stand for several days.

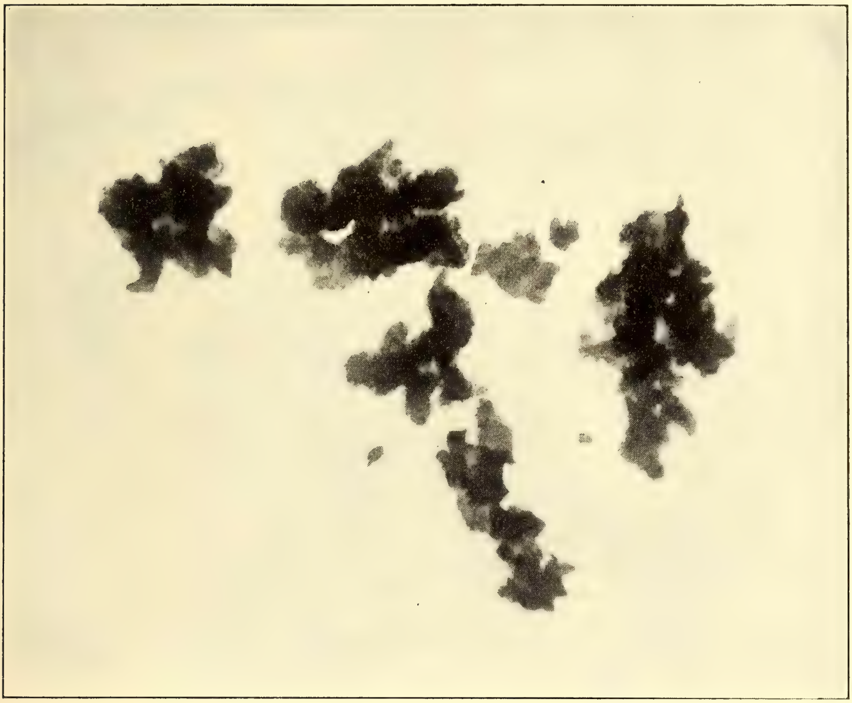

FIG. 1. Photomicrograph of crystal aggregates of endothine red deposited from water.

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[6] The author is indebted to Dr. Hawkins for most of the crude material used in this investigation.

The coloring matter was deposited from water as a mass of crystalline aggregates, as illustrated in figure 1.

The substance, crystallized from alcohol (figure 2), forms glittering "ferruginous"[1] thin plates having a metallic luster. It is readily soluble in pyridine, slightly soluble in cold ethyl alcohol, methyl alcohol, acetic ether, ether, and acetone; very sparingly soluble in cold water, and insoluble in acetic acid, toluene, benzene, carbon tetrachloride, chloroform, carbon disulphide, and petroleum ether. By warming, it is readily soluble in alcohol, but sparingly in water, acetic ether, and acetone. When warmed with glycerine on a water bath a red solution results.

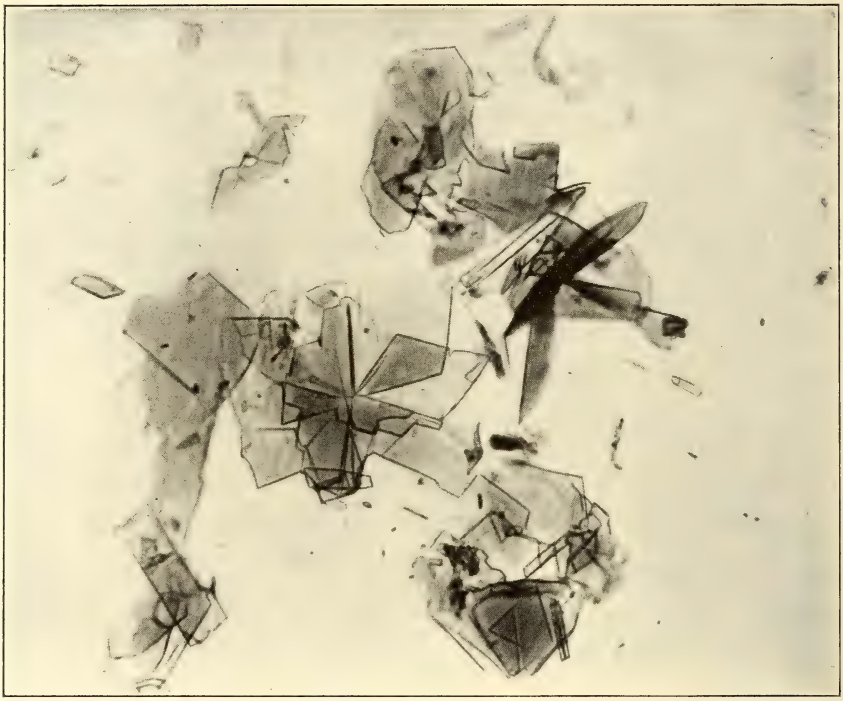

FIG. 2. Photomicrograph of crystals of endothine red purified from alcohol.

Dilute alkalis dissolve it with a violet red color; the addition of dilute acid to this solution changes it to yellow.

Concentrated sulphuric acid gives a "perilla purple" solution.

Cold concentrated nitric acid has no effect on it even after standing 24 hours, but upon gentle warming a faintly colored solution results.

Alcoholic ferric chloride produces with very dilute solution of the pigment a greenish "raw sienna" color; with more concentrated solutions a "dull greenish-black" precipitate is obtained.

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[7] Color descriptions placed in quotations indicate that they were taken from Ridgway, Robert, Color standards and nomenclature. Washington, D. C., 1912.

Alcoholic solutions of sodium and barium acetates yield insoluble sodium and barium salts.

Silver nitrate and Fehling's solution are both reduced, the former even in the cold.

When heated between watch glasses a portion of the substance sublimes without melting, giving rise to a "Brazil red" sublimate. A carbonaceous mass remains in the lower watch crystal.

Combustions were made of separate portions of the pigment, one purified from alcohol, dried at 150°–200° C., and the other purified from water and dried at 100° C. The results are given in table 1.

TABLE 1. *Combustions of Endothine Red*

Samples *A* and *B* from alcohol and samples *C* and *D* from water.

Sample	Weight	CO_2	H_2O	C, Percent	H, Percent	O, Percent
A	.1719	.3467	.0543	55.00	3.53	41.47
B	.1591	.3186	.0509	54.62	3.58	41.80
C	.1572	.3136	.0504	54.40	3.58	42.02
D	.1803	.3628	.0565	54.87	3.51	41.62
Arithmetic mean of four determinations				54.73	3.55	41.72
Required for $C_7H_5O_4$				54.88	3.29	41.83

Since the combustions of the material purified by the two methods and dried at the different temperatures are in excellent accord, it is evident that endothine red contains no water of crystallization.

Diacetylendothine red. Attempts to obtain the acetyl derivative of endothine red by the employment of anhydrous sodium acetate were unsuccessful. The material was, therefore, boiled with acetic anhydride for several hours, and toward the end the excess of reagent was removed by evaporation. On cooling a semicrystalline mass separated, which was filtered with suction, washed with absolute alcohol and ether, and recrystallized several times from warm absolute alcohol. A portion of the acetyl derivative is hydrolyzed to the original pigment when the alcohol contains an appreciable quantity of water. Even when preserved in a sealed tube for several months, darkening of the acetyl compound takes place. This change is rather remarkable when the pigment itself is so stable that cold concentrated nitric acid fails to attack it. The acetyl derivative was dried at 100° C. and combustions were made, with the results shown in table 2.

TABLE 2. *Combusion of Acetyl Derivative of Endothine Red*

Sample	Weight	CO_2	H_2O	C, Percent	H, Percent	O, Percent
A	.2000	.4034	.0720	55.01	4.03	40.97
B	.2007	.4047	.0723	54.99	4.03	40.98
C	.1576	.3217	.0553	55.60	3.93	40.47
Arithmetic mean of three determinations				55.20	4.00	40.80
Required for $C_7H_3O_4(C_2H_3O)_2$				55.67	3.82	40.51

The acetyl derivative forms glistening yellow lath-like crystals which probably belong to the triclinic system[8]. The compound melts with decomposition at about 184°–186° C. (uncorrected). Hawkins and Stevens give 196°–197° C. (uncorrected) as the melting point of a white acetyl derivative of endothine red. Repeated efforts to verify their statement[9] were not successful, since only the yellow derivative described above resulted in all trials.

The number of acetyl groups was ascertained by determining the acetic acid liberated, and the results were confirmed by determining the yield of endothine red recovered on hydrolysis of the diacetyl compound. Three methods were followed in estimating the acetic acid liberated on hydrolysis of the acetyl derivative.

The first method[10] consisted in boiling the compound in an Erlenmeyer flask for several hours with dilute barium hydroxide. The mixture was then acidified at ordinary temperature with phosphoric acid and the acetic acid distilled off. The distillate was treated with an excess of barium hydroxide, evaporated to a small volume and treated with carbon dioxide. The solution was then separated from the excess barium carbonate by filtration and the filtrate evaporated to dryness in a platinum dish. The residue was taken up in carbon dioxide-free water. The barium in solution was determined as sulphate.

For the second method[11] the acetyl derivative was placed in a flask with 30 cc. of alcohol and 2 cc. of sulphuric acid. The mixture was subjected to distillation until two thirds of the liquid had passed over. Fresh alcohol was then added and the operation repeated until the distillate gave no test for acetic acid. The distillate was collected in standard alcoholic potash which was subsequently heated a few minutes to hydrolyze the ethyl acetate formed and finally titrated with standard sulphuric acid.

In the third method[12] the substance was hydrolyzed with dilute sulphuric acid (75 parts of acid to 32 parts of water) on a hot water bath for one half hour, then diluted to 8 volumes and heated on a boiling water bath for 3 hours. The whole process was carried out under a reflux condenser. After 24 hours, the insoluble pigment was filtered off, washed with a little water, dried, and weighed. The filtrate was subjected to steam distillation until about 500 cc. had passed over. The distillate was titrated against standard alkali with phenolphthalein as an indicator. The results for the three methods are given in table 3.

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[8] The author is indebted to Mr. C. M. Smith, of the Insecticide and Fungicide Board, or the examination under the petrographic microscope of endothine red and its diacetyl derivative.

[9] In attempting to clarify this point, Dr. Hawkins assured the writer that the acetyl derivative described in their paper was obtained in small quantities and may have resulted from an impurity present with the pigment.

[10] Herzig, J. Studien über Quercetin und seine Derivate. I. Abhandlung. Monatshefte für Chemie **5**: 72–93. 1884.

[11] Perkin, Arthur George. The determination of acetyl groups. Journ. Chem. Soc. (London) Trans. **87**: 107–110. 1905.

[12] Liebermann, C. Ueber einige früher beschriebene Derivate des Quercetins. Ber. Deutsch. Chem. Ges. **17**: 1680–1684. 1884.

TABLE 3. *Determination of the Acetic Acid Liberated from Acetylendothine Red*

Method Used	Weight of Acetyl Comp.	$BaSO_4$	$C_2H_4O_2$	$C_2H_4O_2$, Percent
1st	.8986	.8122		46.45
2d	.3012		.1425	47.34
2d	.2723		.1275	46.80
3d	.2253		.1029	45.68
Arithmetic mean of four determinations				46.57
Required for				
$C_7H_4O_4(C_2H_3O)$				30.78
$C_7H_3O_4(C_2H_3O)_2$				50.64
$C_7H_2O_4(C_2H_3O)_3$				64.52

The percentage of endothine red in the acetyl compound was determined by weighing the pigment regenerated in the last of the three processes mentioned above for the determinations of the acetyl groups in the compound, as the pigment is only slightly soluble in dilute sulphuric acid. This percentage was also determined by another method[13] which consisted in hydrolyzing the diacetylendothine red in a small volume of acetic acid to which a little sulphuric acid was added. When the mixture was allowed to stand for a short time, red crystals began to separate out. By adding the mixture to several volumes of water and allowing it to stand twenty-four hours the separation was completed.

TABLE 4. *Percentage Yield of Recovered Endothine Red from Acetylendothine Red*

Method Used	Weight of Acetyl Compound	Recovered Pigment	Endothine Red, Percent
1st	.4532	.2938	64.82
2d	.1572	.1014	64.50
2d	.2253	.1457	64.66
Arithmetic mean of three determinations			64.66
Required for			
$C_7H_4O_4(C_2H_3O)$			78.97
$C_7H_3O_4(C_2H_3O)_2$			64.55
$C_7H_2O_4(C_2H_3O)_3$			54.48

The recovered endothine red was obtained as a "Brazil red" crystalline powder whereas the original coloring matter possessed a "ferruginous" color. On this account it was first thought that the recovered pigment had suffered a change in the process of hydrolysis. To decide this point the behavior of the two was studied more in detail. Both gave an "apricot orange" streak and acted in the same way toward such reagents as ferric chloride, dilute alkalis, sodium carbonate, and acids, and showed no differences when examined through the spectroscope. A spectral transmission curve of the pure endothine red is shown in figure 3, and, since the recovered pigment gave a similar curve, no doubt remains that the two compounds are identical.[14]

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[13] Perkin, Arthur G. Luteolin. Part I. Journ. Chem. Soc. (London) Trans. **69**: 206–212. 1896.

Practically none of the violet rays are transmitted; in the blue and green portions of the spectrum there is slightly more transmission, while there is a gradually increasing transmission in the yellow, orange, and red portions.

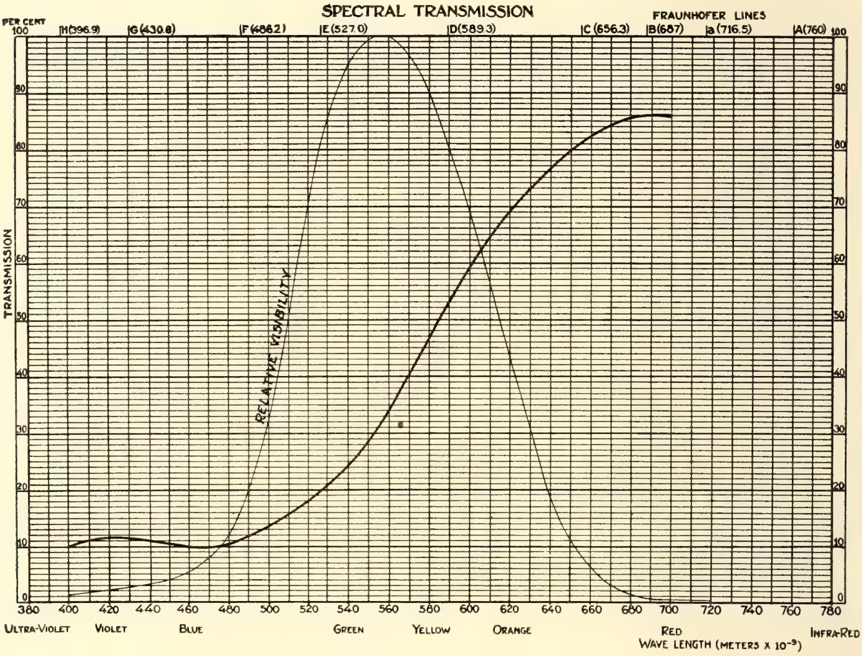

FIG. 3. Curve of percentage of spectral transmission of endothine red in alcoholic solution. Concentration, 0.00475 grams in 1 cc.

A combustion of the regenerated pigment furnished additional evidence of its identity with endothine red.

.1662 gm. gave .3340 gm. CO_2 and .0547 gm. H_2O.
Found C = 54.80, H = 3.68, O = 41.52.
Required for $C_7H_5O_4$ C = 54.88, H = 3.29, O = 40.83.

Molecular weight determination. Endothine red itself could not be used for the determination of its molecular weight on account of its slight solubility in solvents used for such determinations. It was found necessary, therefore, to use the diacetylendothine red for this purpose. The results with benzene as the solvent (K 2700) are reported in table 5.

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[14] The writer is indebted to Mr. I. C. Priest, of the Colorimeter Laboratory, Bureau of Standards, for this work.

TABLE 5. *Molecular Weight Determination of Diacetylendothine Red in Benzene*

Sample	Weight of Sample	Weight of Solvent	Elevation of the Boiling Point	Molec. Wt.
A	.0741	15.0351	.055°	241.9
B	.0848	"	.085	179.1
C	.0881	"	.065	243.4
D	.2470	"	.205	216.3
Arithmetic mean of four determinations				220.1
Required for $C_7H_3O_4(C_2H_3O)_2$				237.07

Disodium endothine red is formed on adding alcoholic sodium acetate to a boiling alcoholic solution of the coloring matter. It forms a "brownish olive" powder which is insoluble in alcohol, but readily soluble in water in which it gives a violet red solution. Apparently it undergoes no decomposition on boiling with water. The compound was dried at 100° and analyzed by heating in a muffle furnace for 6 hours.

.1600 gm. gave .0720 gm. Na_2CO_3. Found Na = 19.55.
.2106 gm. gave .0949 gm. Na_2CO_3. Found Na = 19.57.
$C_7H_3O_4Na_2.2H_2O$ requires Na = 19.77.

The water of crystallization was not determined. At the time of determination the possibility that the compound contained water of crystallization had not presented itself, and lack of material prevented further work on this point later.

Monobarium endothine red, $C_7H_3O_4Ba.2H_2O$, forms an olive-green felt-like mass when alcoholic barium acetate is added to a boiling alcoholic pigment solution. It is insoluble in cold water and in alcohol.

.2714 gm. gave .1638 gm. $BaCO_3$. Found Ba = 42.06.
.2823 gm. gave .2011 gm. $BaSO_4$. Found Ba = 41.92.
$C_7H_3O_4Ba.2H_2O$ requires Ba = 42.35.

THEORETICAL CONSIDERATIONS

In calculating the empirical formula for endothine red from the elementary analysis, three formulas seemed to give figures which are in fairly close agreement with the experimental values. *There seems to be no doubt that* $C_7H_5O_4$ *represents the true formula of this new coloring matter.* Table 6 gives the data from which the selection of the assigned formula was made.

The results of this investigation indicate that endothine red contains two hydroxyl groups and has acid properties which enable it to decompose alkali salts. Efforts to prepare an ester by prolonged digestion with alcohol and sulphuric acid in the usual manner failed. The acid properties can not, therefore, be ascribed to the presence of a carboxyl group. In this connection it is interesting to refer to the work of Perkin[15] which deals with

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[15] Perkin, Arthur George. A reaction of some phenolic coloring matters. Journ. Chem. Soc. (London) Trans. **75**: 433–454. 1899.

TABLE 6. *Data Leading to the Selection of $C_7H_5O_4$ as the Formula of Endothine Red*

Found	Required for		
	$C_7H_5O_4$	$C_9H_7O_5$	$C_{14}H_{11}O_8$
C H 55.00 3.53 54.62 3.58 54.40 3.58 54.87 3.51 mean 54.73 3.55	C H 54.88 3.29	C H 55.27 3.61	C H 54.77 3.61
Acetyl Derivative C H 55.01 4.03 54.99 4.03 55.60 3.93 mean 55.20 4.00	$C_7H_4O_4(C_2H_3O)$ = 55.27 C 3.61 H $\boxed{C_7H_3O_4(C_2H_3O)_2 = 55.67\text{ C }\ 3.82\text{ H}}$ $C_7H_2O_4(C_2H_3O)_3$ = 55.89 C 3.97 H	$C_9H_6O_5(C_2H_3O)$ = 55.67 C 3.82 H $C_9H_5O_5(C_2H_3O)_2$ = 55.89 C 3.97 H $C_9H_4O_5(C_2H_3O)_3$ = 56.05 C 4.08 H	$C_{14}H_8O_8(C_2H_3O)_3$ = 55.40 C 3.96 H $C_{14}H_7O_8(C_2H_3O)_4$ = 55.56 C 4.03 H $C_{14}H_6O_8(C_2H_3O)_5$ = 55.68 C 4.09 H
Yield of Regenerated Pigment 64.82 64.50 64.66 mean 64.66	$C_7H_4O_4(C_2H_3O)$ = 78.97 $\boxed{C_7H_3O_4(C_2H_3O)_2 = 64.55}$ $C_7H_2O_4(C_2H_3O)_3$ = 54.48	$C_9H_6O_5(C_2H_3O)$ = 82.27 $C_9H_5O_5(C_2H_3O)_2$ = 69.89 $C_9H_4O_5(C_2H_3O)_3$ = 60.74	$C_{14}H_8O_8(C_2H_3O)_3$ = 70.08 $C_{14}H_7O_8(C_2H_3O)_4$ = 64.62 $C_{14}H_6O_8(C_2H_3O)_5$ = 59.37
Acetic acid from Acetyl Derivative 46.45 47.34 46.80 45.68 mean 46.57	$C_7H_4O_4(C_2H_3O)$ = 30.78 $\boxed{C_7H_3O_4(C_2H_3O)_2 = 50.64}$ $C_7H_2O_4(C_2H_3O)_3$ = 64.52	$C_9H_6O_5(C_2H_3O)$ = 25.32 $C_9H_5O_5(C_2H_3O)_2$ = 43.01 $C_9H_4O_5(C_2H_3O)_3$ = 56.08	$C_{14}H_8O_8(C_2H_3O)_3$ = 41.81 $C_{14}H_7O_8(C_2H_3O)_4$ = 50.53 $C_{14}H_6O_8(C_2H_3O)_5$ = 58.03
Regenerated Pigment C H 54.80 3.68	C H 54.88 3.29	C H 55.27 3.61	C H 55.77 3.61
Molecular weight of Acetyl Compound 179.1 (?) 241.9 216.3 243.4 mean 220.1	$C_7H_4O_4(C_2H_3O)$ = 195.06 $\boxed{C_7H_3O_4(C_2H_3O)_2 = 237.07}$ $C_7H_2O_4(C_2H_3O)_3$ = 279.09	$C_9H_6O_5(C_2H_3O)$ = 237.07 $C_9H_5O_5(C_2H_3O)_2$ = 279.09 $C_9H_4O_5(C_2H_3O)_3$ = 321.10	$C_{14}H_8O_8(C_2H_3O)_3$ = 433.14 $C_{14}H_7O_8(C_2H_3O)_4$ = 475.15 $C_{14}H_6O_8(C_2H_3O)_5$ = 517.17
Na from Sodium Derivative 19.55 19.57 mean 19.56	$C_7H_4O_4Na$ = 13.16 $C_7H_3O_4Na_2$ = 23.38 $\boxed{C_7H_3O_4Na_2, 2H_2O = 19.77}$ $C_7H_2O_4Na_3$ = 31.54	$C_9H_6O_5Na$ = 10.59 $C_9H_5O_5Na_2$ = 19.27 $C_9H_4O_5Na_3$ = 26.47	$C_{14}H_9O_8Na_2$ = 13.13 $C_{14}H_8O_8Na_3$ = 18.52 $C_{14}H_7O_8Na_4$ = 23.32
Ba from Barium Derivative 42.06 41.92 mean 41.98	$C_7H_3O_4Ba$ = 47.63 $\boxed{C_7H_3O_4Ba, 2H_2O = 42.35}$	$C_9H_5O_5Ba$ = 41.57	$C_{14}H_9O_8Ba_2$ = 47.54

the acid properties of coloring matters of the anthraquinone, flavone, xanthone, and ketone groups. He says: "With the exception of morin and rhamnazin, no coloring matter of known constitution is markedly acidic unless it contains two hydroxyls in the ortho-position relatively to one another, and consequently has strong dyeing properties." Further evidence that the two hydroxyls are in the ortho-position in endothine red was obtained by fusing the compound with potash. By heating the pigment to 200°–250° C. for half an hour with ten times its weight of potassium hydroxide, dissolved in a little water, a phenol portion was obtained, which gave a green coloration with ferric chloride. The fusion was carried out several times, and at no time could the phenol be isolated in sufficient quantities for further identification. It is probable, however, that it is pyrocatechin or a member of this group since this class of phenols gives a green color with ferric chloride. A red acid portion of the potash fusion gave a greenish "raw sienna" color with ferric chloride and appeared to consist partly at least of the unchanged pigment.

The foregoing considerations justify the conclusion that endothine red is probably related to the members of the pyrocatechin group. The exact constitution of a side chain, if such exists, and further confirmation of the meager evidence at hand will, it is hoped, be the subject of another investigation.

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Washington, D. C.

GROWTH AND VARIABILITY IN HELIANTHUS[1]

H. S. Reed

The growth and final size of organisms are known to exhibit greater or less deviations from the mean for any group or population.

Growth may be considered as a function of two variables. The first is the genetic constitution of the individual, and the second is the resultant of all those factors which make up what is commonly called the environment of the organism. The factors of the first group are essentially *internal,* those of the second group, essentially *external.*

The variability in the growth rate and in the final size of the organism is a question of keen interest. Much attention has been paid in the past to the extent and nature of the variability in mature organisms, but few studies have been made upon the question of variability during the grand period of growth.

A brief consideration of the problem convinces one that it is not sufficient to plant seeds of known weight and to measure the mature individuals at the end of the growing season. Many questions must necessarily be unanswered by the result of such determinations. For example, one immediately wishes to know whether the smaller individuals in the population have been smaller from the beginning, or whether they were normal in size for a time and came to maturity early; while others of the same, or of similar size, continued to grow later and finally attained more than average size.

While we recognize variability in the size of plants, we also need to know whether a plant or a group of plants exhibits a deviation from the theoretical mean of the population greater or less than that to be expected upon the basis of pure chance; in other words, are the variations purely chance variations, such as might be due to the effect of purely casual factors of environment, or are they so characteristic as to indicate that they are due to something else?

The studies embodied in the present paper are based on measurements of a group of sunflowers (*Helianthus annuus* L.) grown for the purpose on the grounds of the Citrus Experiment Station, Riverside, California. They were grown on a small piece of tolerably uniform soil to which water, sufficient to maintain satisfactory soil moisture conditions, was applied every seven days. The plants grew from the middle of May to the middle of August, during a time when heat and light were ample for plant growth. As soon as the plants had reached an average height of more than ten centimeters, sixty of the normal-appearing plants were selected at random throughout the small plot and marked with suitable labels. (Two plants were subsequently discarded on account of accident.) Each plant was marked with India ink at a distance of ten centimeters below the growing tip. This mark served as a point from which further measurements were made. Adjacent plants were removed from the vicinity of those selected so that a space of at least 20 centimeters intervened between any plant and its nearest neighbor. In short, environmental conditions were made as nearly uniform for the individuals in this small group as it was feasible to make them under field conditions. In the latter part of July the terminal buds began to develop into blossoms, and coincidentally the plants ceased to elongate.

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[1] Paper 57, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

The population contained both branched and unbranched individuals. The branched form usually produces a head on the apex of each branch, whereas the unbranched form produces only one head and that develops from the apical bud of the stem. The branching habit is regarded by Shull (1908) as a Mendelian character. One important difference should be noted between the plants described by Shull and those here considered, *viz.*: Shull's plants branched from the lower nodes of the stalk, while these branched only from the upper nodes.

Church (1915) regards the branched form as a mutant of the unbranched and believes that it is the oldest mutation on record.

This mixture of branched and unbranched stems is not thought to affect the validity of the measurements upon which the present study is based, since only 17 out of the 58 plants were branched and the average heights of the two classes at maturity were close enough together to be within the range of the probable error. The average growing period of the branched plants was 4.6 days longer than that of the unbranched plants. The number of heads produced by the branched plants ranged from 3 to 13.

Rate and Amount of Growth

The measurements made on the 58 plants are given in table 1, which shows the heights of the individual plants at seven-day intervals, dating from the time when each plant was marked 10 centimeters below the growing tip and continuing until no further elongation occurred. On the 84th day, when the last measurement was taken, the plants averaged 254.5 centimeters high to the upper side of the head, with a range from 164 centimeters to 339 centimeters.

The measurements of all plants have been assembled for study *en bloc* (table 1). The mean height of the plants at seven-day intervals is shown by figures in table 2, together with the standard deviations and coefficients of variability. The mean height of plants at seven-day intervals is shown graphically in figure 1.

The data show that the plants grew slowly at first, reached the maximum growth rate between the 35th and the 42d days, *i. e.*, about the middle of the grand period of growth; the rate then declined as the end of the grand period of growth was approached.

A great decline in the growth rate of the plants began to appear as the flower bud on the apex of the stalk began to be differentiated. As the "head" developed, the growth of the stalk became slower, showing agreement with the condition which Pearl and Surface (1915) found when the tassel is formed on maize. After the flowers of the composite "head" had been pollinated, there was no further elongation of the stalk. It is probable that from this time on the growth forces of the plant are devoted to seed formation instead of to elongation of the stalk. Thus, variability in the time of blossoming may, and undoubtedly does, influence the grand period of growth and the total growth of this plant. When the flowers of the head have been pollinated, the head, which previously stood erect, becomes pendant. The floral surface, which is uppermost during the prepollination process, is lowermost during the post-pollination period.

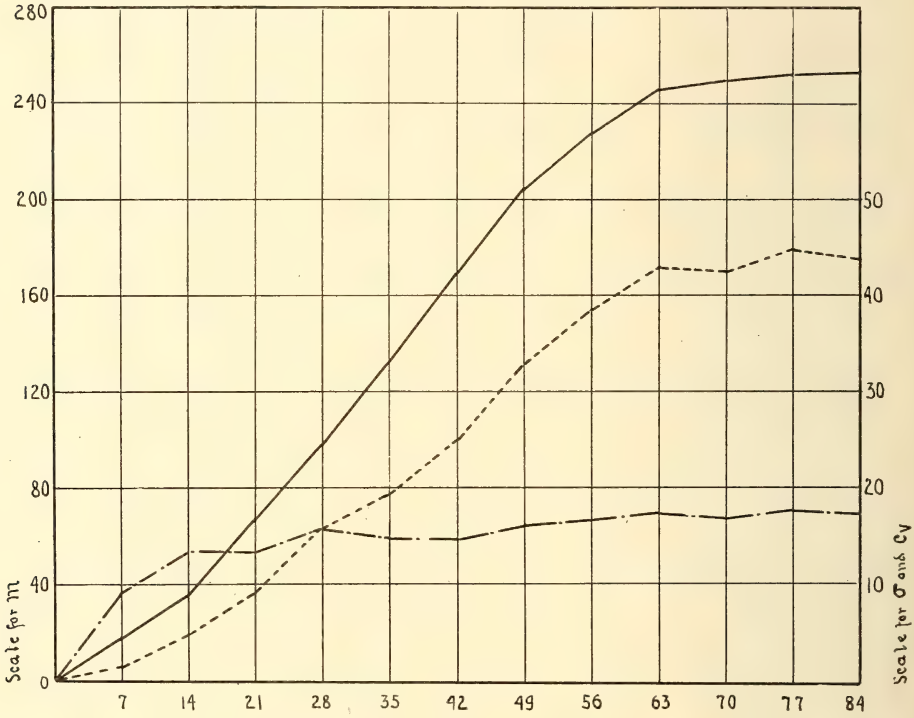

FIG. 1. Curves showing progressive changes in mean height, standard deviation and coefficient of variability of sunflower plants during the grand period of growth.

——————————— Mean
– – – – – – – – – – – – – Standard Deviation.
—·—·—·—·—·—·— Coefficient of Variability.

Variation in the Growth and Final Size of Plants

The growth rate of this group of plants has been studied and found to agree closely with that of an autocatalytic reaction in which one of the products catalyzes the reaction (Reed and Holland, 1919). We may, therefore, turn from the question of the growth rate of the population as a whole, to consider some problems of growth and variability relating to smaller groups within the large group. We should attempt to discover whether the large and the small members of the group differ from each other in their growth rates as well as in their final size; to be specific, whether the small plants grew slowly during the entire season, or whether they grew rapidly during their early history and came sooner to maturity.

Closely related to this, is the question of relative position in the population during the whole period of growth. Do plants which are undersized at the start grow rapidly enough to get into higher groups as they become older, or do they remain undersized to maturity?

Another question, and one which is also wrapped up in the questions previously propounded, is that of the nature of the variability of the groups. Are their variations those which are due to mere chance of environment, or are they due to some other cause, which may be referred to the genetic constitution of the plant?

For purpose of study, it is convenient to classify the sunflower plants upon the basis of their size each week during the growing season. The quartile[2] was used because it gave enough plants in each class to offset minor errors, such as are bound to occur in quantitative work. In order to make comparisons more valid, the number of plants in each quartile was fixed at the outset and adhered to throughout. The divisions were as follows: the first and third quartiles contained 15 plants each; the second and fourth 14 plants each. The first quartile contained the 15 shortest plants at each time of making measurements; the second quartile contained the 14 next taller; the third quartile contained the 15 next taller and the fourth quartile contained the 14 tallest plants. In some few cases, one or more plants fell on the quartile boundaries. It was then necessary to assign them to one or the other adjacent quartile. This was done in as impartial a manner as possible, in order that the assignments might have a purely random nature. The boundaries of the quartiles never varied widely from the limits obtained from the value $x/\sigma = 0.675$.

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[2] In using the term "quartile" to designate a portion of the population, I am aware of some danger of confusion of terms. Strictly speaking, a quartile is a point so situated on the base of a frequency polygon that one fourth of the individuals lie on one side and three fourths on the other. Yet there seems to be some authority for using it to designate one fourth of the area of the frequency polygon (*cf.* use of term "quintile" by Pearl and Surface, 1915). In this paper the term "quartile" will be used to designate one fourth of the population.

THE QUARTILE DEVIATIONS OF PLANTS STARTING IN THE SEVERAL QUARTILES

The classification into quartiles having been made, it was of interest to study the behavior of plants starting in a given quartile. Tables 3 to 6 show the quartile position, at each observation, of the plants which were in a given quartile on the 14th day. The measurements of the plants on the 7th day were grouped so closely about the mean that the limits of the quartile range would have been far inside the errors of measurement. The average height for the population on the 7th day was 17.93 cm. with a standard deviation of only 1.62 cm.; only 10 plants fell outside of a range of 16 to 19 cm.

A brief inspection of table 3 will make clear the method of ascertaining the quartile positions of the plants at successive intervals of time. It is there shown that there were 15 plants in quartile I on the 14th day. On the 21st day, 12 of these were still in quartile I, two were in quartile II, and one in quartile III. The mean quartile position of these plants on the 14th day was obviously 1.000, on the 21st day it was 1.226. As time went on, these 15 plants were gradually more widely distributed through the several quartiles, and at the close of the growing period the mean quartile position was 2.266 with the comparatively large standard deviation of 1.18.

A more concrete idea of the distribution may be obtained by reference to the figures in the next to the last column of the table. There were 10 distributions, each containing 15 observations, or a total of 150. From the figures in the "total" column, it is seen that 68 observations fell in quartile I, 31 in quartile II, 28 in quartile III, and 23 in quartile IV. The same values are expressed in percentaegs of the total number of observations in the last column.

The observations on the quartile positions made on the 14th day were excluded from the computations, since they were, by hypothesis, in a certain quartile on that day, and we could not assume that the laws of simple sampling would apply if they were included.

If we note the total number of observations in the various quartiles, it will be seen that the plants which were small on the 14th day tended to remain small to the end, half of the total number of observations being located in the first quartile and the remainder being scattered through the other three quartiles.

An inspection of table 4 shows the behavior of plants which were in the second quartile on the 14th day. Here again it will be seen that plants which started in this quartile tended to remain in it. Of the total observations, 37 percent were in the second quartile. In comparison with the plants which started in the first quartile, there is a somewhat greater tendency toward dispersion. It might be argued, and with justice, that such a tendency might logically be expected, since the plants starting in the first quartile would be dispersed only in one direction, *viz.*, upward, while those which started in the second quartile might be dispersed either upward or downward on the scale. Be that as it may, however, the plants which started in the second quartile showed a greater tendency to deviate toward a higher quartile than toward the lower one, and those starting in the third quartile showed a greater tendency to deviate toward a lower than toward a higher quartile.

Tables 5 and 6 show the quartile positions at successive observations of plants which started in the third and fourth quartiles respectively. It will economize time and space, however, to bring the observations on all the distributions together in one table and there to discuss the characteristics of the several quartiles.

Table 7 presents a summary of the observations on the quartile distributions of all the plants starting in the various quartiles. It shows, for the plants which started in the various quartiles, the number and percentage of observations falling in the several quartiles at successive dates. The first line shows the number and percentage of the observations falling in the different quartiles for the plants which started in the first quartile, and so down the table. The table brings out still more plainly the tendency for a group of plants to remain in or near that quartile in which they started.

By use of a method of comparison which has been given by Pearl and Surface (1915), it is possible to obtain a concrete, definite, quantitative expression of this tendency for plants to retain the quartile position with which they started.

The measure of this tendency calls for the consideration of certain aspects of the theory of probability. If nothing but pure chance were operating to determine the quartile positions of these plants, it is evident that they would fall in one quartile as often as in another. The results would, consequently, be comparable to those obtained by making an equal number of throws of four-sided dice. After the allocation of the plants to quartiles, they were measured ten times, consequently there were 150 observations on the quartile positions of plants starting in the first and third quartiles and 140 observations on those starting in the second and fourth quartiles. It will be necessary, first, to consider what the results would have been if nothing but pure chance were operating to determine the distribution of the plants. It is well known that the chances of success of an event where p = the chance of success and q = the chance of failure, are $p \cdot q = 1$. In this case, the chance of success would be very close to $\frac{1}{4}$ and that of failure to $\frac{3}{4}$.

The theoretical standard deviation of the percentage of successes in n events would be $\sigma = \sqrt{p \cdot q / n}$ which will give the standard deviation from the theoretical mean percentage, provided we are dealing with a purely chance distribution, such as throwing four-sided dice.

The standard deviation of the theoretical mean for quartile I is, therefore,

$$\sigma = \sqrt{\frac{24.94 \times 75.06}{150}} = 3.533,$$

and for quartile II is

$$\sigma = \sqrt{\frac{24.56 \times 75.44}{140}} = 3.638,$$

and similarly for the other quartiles as shown in the next to the last column in table 7.

A range of $\pm 3\sigma$ from the theoretical mean ought to include all, or nearly all, of the observations; therefore there should be few if any observations above 35 percent or below 15 percent if the distributions were governed by pure chance. An examination of table 7 shows that, as a matter of fact, the percentages of observations falling within a given quartile do deviate more widely than the limits to be expected under the laws of pure chance. As in the case of the maize plants studied by Pearl and Surface (1915), the greatest positive deviations were in that quartile in which the sunflower plants started, and the greatest negative deviations were in the quartile farthest from that in which the plants started. Furthermore, it will appear that plants starting in quartiles I and IV deviated more widely from their respective theoretical means than plants starting in quartiles II and III deviated from their means. From this it appears that plants which start as small, medium, or large individuals in a population, though they vary more or less, tend to stay in or near the class in which they started.

We may next attempt to get a concrete expression of the observed deviations which have been discussed above in a general way. We shall find that the root-mean-square deviation of the observed percentage values (or their actual standard deviation) from the theoretical mean is an excellent measure of their tendency to deviate from the theoretical results of simple sampling, and that it is a measure of those influences, other than chance, which determine the *locus* of the given group of plants in the population, because it shows how widely the plants starting in any given quartile depart from the position which would have been most probable according to the laws of pure chance.

We may proceed to compare the theoretical standard deviation of the mean with the observed deviations of the percentage from these theoretical mean percentages. This is conveniently done by obtaining the actual standard deviation from the theoretical mean for the plants, starting in the various quartiles. The deviations of the several percentages from their theoretical mean percentages are obtained and each is squared, then the actual standard deviation equals $\sqrt{\Sigma d^2/n}$. The values are shown in the last column of table 7.

Inspection of the results shows at once that the actual standard deviations are considerably greater than the theoretical. This can only indicate that there is some agency operating to cause variability in height in excess of that to be expected upon the basis of pure chance. Moreover, regarding the higher values obtained for the plants in quartiles I and IV, we must conclude that these agencies are more influential on the plants varying most greatly from the mean height of the population on the 14th day. It will be seen that the actual standard deviation is an extremely useful constant, since it measures the influence of those factors (aside from pure chance) which determine the relative height of the plants during their grand period of growth.

Before discussing the growth characters further, it will be well to examine their behavior when studied from the point of view of the growth rate of plants ending in the several quartiles.

THE QUARTILE DEVIATIONS OF PLANTS ENDING IN A GIVEN QUARTILE

The population was divided into four groups according to their heights at the end of the growing season. On the 84th day 15 plants were in quartiles I and III, and 14 plants in quartiles II and IV respectively. The quartile positions of these plants were then determined on each of the days of observation.

Tables 8, 9, 10, and 11 show the number of plants in the several quartiles at each measurement and the mean quartile positions. The plants which ended in any given quartile were distributed through all the quartiles on the 14th day, yet even then the tendency to be grouped in or near the quartile in which they ended was manifest. It will be noted that on the 14th day the mean quartile position for the plants ending in the several quartiles was nearly the same and that it was near the mean quartile position (2.5) of the population as a whole. By the 42nd day the plants ending in quartiles I and IV had come within less than a quartile of reaching their final position, while those ending in quartiles II and III were very close to their final position. In each case the percentage of observations falling in the particular quartile in which the plants ended was somewhat higher than similar values for plants starting in the several quartiles as given in tables 4 to 7, inclusive. From this it appears that the final quartile position of the plants was a better means of judging their average position during the grand period of growth than the initial position. The opposite relation in the case of maize plants was found by Pearl and Surface (1915). It may be remarked in passing that the plants ending in a given quartile showed little change in the mean quartile positions after the 42d day.

Turning now to table 12, where the data are more compactly assembled, it will be noted that much the same tendency to deviate is shown by these figures as by those in table 7. The observations show that the plants were more frequently in the quartiles in which they finished than in any other.

On the basis of pure chance the observations should fall between 15 and 35 percent approximately, yet only 6 out of 16 observations obey this law. This tendency was especially pronounced in the case of plants which ended in quartiles I and IV, in other words, in the extreme classes. This tendency naturally became more pronounced as growth progressed, so there were no changes in the quartile positions of the plants in the last part of the season. This seems to indicate that the plants, as a rule, had a uniform rate of growth, and that the plants which were small at the end of the season were in that class because they had an inherent tendency to smallness all through the season. Conversely, the plants in the highest group were not there because they were mediocre plants which grew longer than the rest, but because they were superior all through the season.

Of the 150 observations made on the plants ending in the first quartile, 67 percent were in that quartile (table 8) and 58 percent of the 140 observations made on the plants ending in the fourth quartile were in that quartile (table 11).

As in the former comparisons, we see that plants which fell into a given class at the end of the season showed a tendency to group themselves in or near that class from the outset. Table 12, which assembles the data of the four preceding tables, affords a convenient means of making comparisons. As in table 7, the actual standard deviation shows that the height of the plants varied more widely than would be the case if their heights were determined by pure chance. These relations make it quite evident that the height of any given group of these plants is not determined by such casual factors as variations in soil, water, light, and other external factors, since they would produce a more nearly random quartile distribution. It seems, on the contrary, that the quartile distributions of the measurements of a plant are determined by internal, inherited factors which operate so strongly and characteristically that they outweigh the influence of casual factors and assert their dominant influence in the behavior of the sunflower. In short, there appears to be some agency at work on the plants which produces deviations from their theoretical means greater than those which are to be expected upon the basis of pure chance, and this influence appears to be most effective on the plants in the extreme classes. The attempt will be made, in a subsequent paragraph, to elucidate somewhat the nature of this influence which manifests itself in the quartile positions of these groups of the population.

It may be of interest, especially for those not familiar with the use of the above described methods, to examine the actual figures. It might be thought that the classes are too broad to reveal actual differences, or that rapid growth in the first part of the season would set a plant so far ahead of others that it would stand in an upper quartile, though making smaller growth subsequently. I have thought it worth while, therefore, to present in table 13 the successive increases in mean height of the plants ending in the various quartiles. Inspection of the table shows that after the 14th day the relative rates of growth are consistently larger in the higher quartiles. In other words, the plants which were largest at maturity were superior all through the season.

CHANGES IN THE QUARTILE POSITIONS OF THE PLANTS DURING THE GRAND PERIOD OF GROWTH

Reference has previously been made to certain tendencies of the plants in the different quartiles either to alter or to maintain their relative positions during growth. Figures 2 and 3 show graphically the changes in relative position at successive measurements, for the plants starting, and for those ending, in the various quartiles.

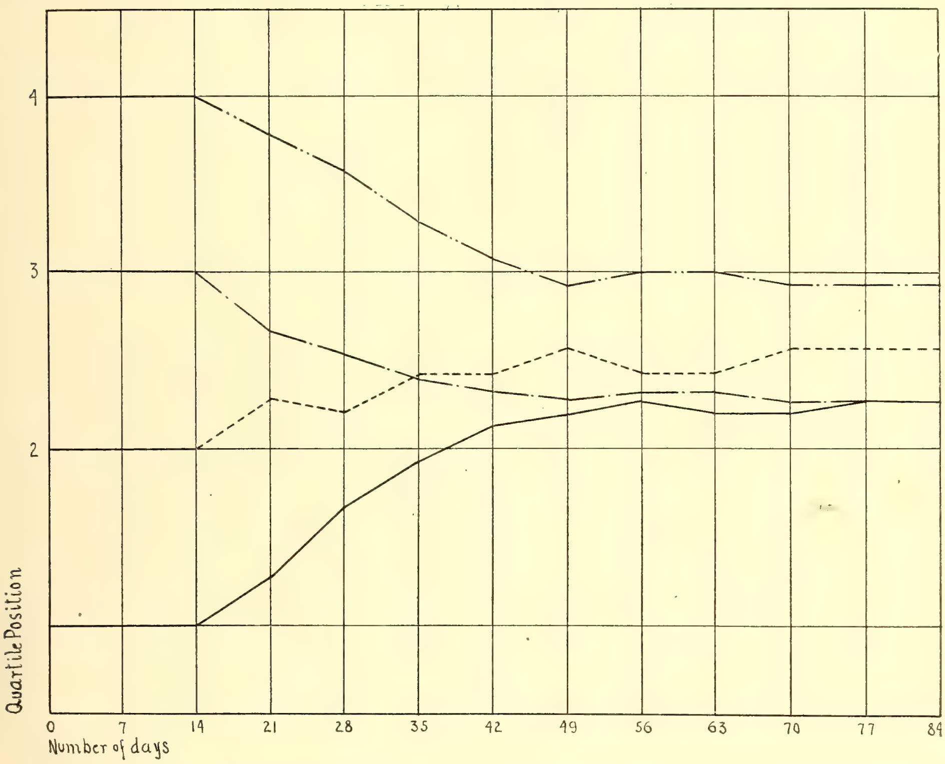

FIG. 2. Curves showing changes in mean quartile positions of plants starting in the several quartiles.

———————— Plants starting in first quartile.
– – – – – – – – " " " second "
—·—·—·—·—·— " " " third "
—··—··—··—··— " " " fourth "

It will be evident, in spite of the changes, that plants tend to stay in or near the quartile in which they start, especially after the middle of the grand period of growth.

GROWTH RATES AND VARIATIONS OF INDIVIDUAL PLANTS

The investigation of the characteristics of these plants was carried farther by extending it to the individuals instead of studying them in groups. The mean quartile position and its standard deviation for each plant in the population have been determined as a basis for this study. Just as in the case of the larger groups, the mean quartile position of a plant gives a measure of its relative size during the entire growth period. A plant which is constitutionally inferior will have a mean quartile position near I, and those which have the opposite tendency will have a mean quartile position near IV, but by the method of grouping none will be below I and none above IV.

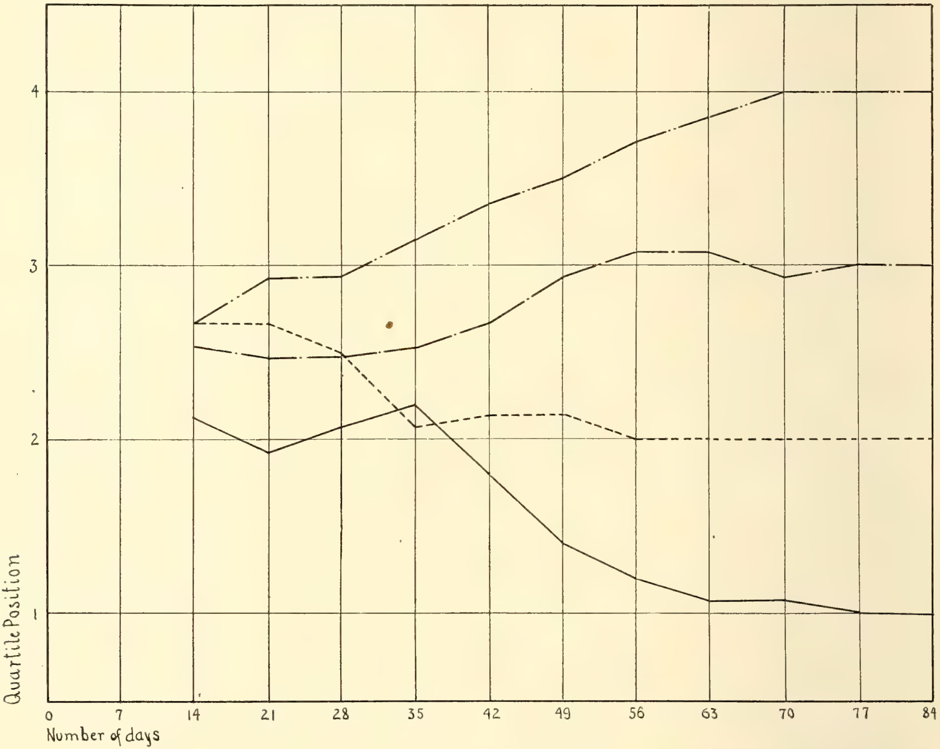

FIG. 3. Curves showing the changes in mean quartile positions for plants finishing in the several quartiles.

——————— plants finishing in first quartile.
– – – – – – – – – – " " " second "
—·—·—·—·—·— " " " third "
—··—··—··—··— " " " fourth "

These values have been determined and are presented in classified form in table 13, where they can be studied more easily than if given for each plant in serial order. The class range has been taken at 0.75 quartile.

The number of plants in the several classes is 12, 15, 17, and 14, and indicates a fairly good random distribution with a tendency to grouping in the middle classes. The average quartile position of each class is not far from the different mid-class values, a fact which may be taken as further evidence of random distribution.

The facts concerning these plants will be brought out by consideration of the means and standard deviations of the positions. Taking the four average means at the bottom of table 14 and considering the number of plants as frequencies for these values, we find that their mean is 2.552 ± .101, and their standard deviation in class units is .8005 ± .0743. Since a class unit is .75 of a quartile the standard deviation in quartiles is found by multiplying this by .75, *viz.*, .6004 ± .0557.

The standard deviation in class units deserves further consideration. It measures the variation which the class units would have if, without reference to grouping, they were arranged in serial order, *i. e.*, as 1, 2, 3, 4. A simple algebraic calculation will show that the standard deviation of the first n numbers is

$$\sigma = \sqrt{\frac{n^2 - 1}{12}} .$$

In this case, $n = 4$, therefore, $\sigma = 1.12$. Now it was found above that the actual standard deviation was .8005 ± .0743. This is somewhat below the theoretical standard deviation, even with the addition of three times its probable error. This relation may be taken to indicate that the variates under discussion (the mean quartile positions) are fairly evenly distributed in the several classes, but that they scatter less widely from the middle classes than would be expected upon the basis of pure chance.

It now becomes possible to point out a fact of fundamental physiological importance, derived from these mathematical relations. Since the means of the quartile positions are so nearly distributed by the law of probability, it seems logical to conclude that the causes of this condition are also distributed by the laws of chance. For example, in table 14, the first average, 1.333, owes its position to the same cause which determined the position of any other average. In other words, the smaller relative size of these plants is an expression of the same definite cause as the relatively larger size of any of the other groups in this population.

Perhaps it is well to call attention here to the fact that the discussion in the above paragraph relates strictly to the *mean quartile distributions* (average relative size) of the plants and not to the *quartile distribution of the measurements of a plant* through the grand period of growth. The latter values are clearly not determined by chance, since under that condition their standard deviation would be .1988 instead of .8005.[3]

It will be profitable to see if we can discuss the probable nature of the causes which determine the relative sizes of the plants without pushing the mathematical analysis too far. Pearl and Surface (1915) found evidence that the height of the maize plants they studied was governed by Mendelian factors. We may with propriety inquire whether the relationships of the relative heights of the sunflowers give similar evidence.

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[3] For the means of arriving at this value, the reader is referred to Pearl and Surface (1915), pp. 151–153. Mention should be made that these writers have laid all subsequent students of this subject under obligation for their illuminating discussions.

If we assume that there is one pair of allelomorphic characters for height, we should have in the F_2 generation, four classes, *AA*, *Aa*, *aA*, and *aa*, each of equal frequency. In such a case, the distribution of the relative heights of the plants should be nearly regular. Also, since the plants in the end classes bearing the factors *AA* and *aa* are homozygous for the theoretical height-factor, they should be less variable than the heterozygous plants in the intermediate classes. We may examine the data to see if either of these assumptions can be supported.

It was pointed out above that the mean quartile distributions are fairly evenly distributed in the several classes, though there is a slight tendency for them to be grouped in the middle classes. A smoother distribution might have been obtained by study of a larger population.

As a measure of the relative variability of the mean quartile positions we may refer to the standard deviations obtained in table 14. The values for the first and fourth classes are .502 and .505 respectively, while those of the intermediate classes are .793 and .786. Certainly there is a significance about these results which seems to warrant the assumption that the plants in the extreme classes are less variable about their mean relative height than those in the intermediate classes.

Objection may be raised to this assumption, since the sunflowers were not known to be the F_2 progeny of a pair of individuals, and to the assumption of a definite ratio. In the work frequently quoted in this article, Pearl and Surface (1915) have shown, however, that this is just what might be expected in any population in which independent Mendelian factors are distributed at random, since in such a population, where open fertilization occurs, the individuals will have the same factor constitution as individuals in the segregating generation. If we assume the presence of two factors for height, we should expect such a result as that obtained. There may be more than two factors for height, but the assumption of a larger number need not invalidate the argument.

Attention may be called to a conclusion drawn from a study of the growth constants of these plants (Reed and Holland, 1919), wherein it was pointed out that the growth constants of plants ending in the several quartiles were practically identical. Differences in the mean relative heights are evidently determined by some internal factor other than differences in the growth constants.

SUMMARY

1. The growth and variability of a small group of sunflower plants, grown under tolerably uniform field conditions, was measured by taking weekly records of the height of each plant during the grand period of growth.

2. An analysis of the growth and relative superiority of the plants has been made to determine (*a*) whether the inferior plants grew slowly during the entire season, or whether they grew rapidly during their early history and came sooner to maturity; and (*b*) whether variability in size of individuals, or of groups, was due to mere chance of environment or to some inherited factor.

3. The quartile class was adopted as a basis for classifying the population into groups. The number of plants in each quartile was fixed at the outset and adhered to through the entire study.

4. Plants which started in a given quartile showed a well marked tendency to remain in that quartile during the entire grand period of growth. Plants which were small at maturity were generally small from the beginning, those which were large at maturity had a well-marked superiority from the start.

5. The actual standard deviations of the observed percentage values of the quartile positions of plants departed so widely and consistently from the theoretical standard deviations of the mean percentages that it seems quite certain that there was some agency operating to cause variability in height in excess of that to be expected upon the basis of pure chance. The assumption is made that the relative size of plants is dependent upon internal genetic factors, rather than upon external casual factors.

6. The mean quartile position of each plant was taken as a measure of its relative size during the grand period of growth. The frequency of the mean quartile positions indicated a good random distribution, though there was a slight tendency toward grouping in the mid-classes. It seems probable, therefore, that the smaller relative size of the plants in the lower classes is an expression of the same definite factor as the relatively larger size of any of the other groups in this population.

7. There was evidence that height was determined by factors which were distributed at random through the population. It was found that the distribution of the relative heights of the plants was nearly equal. Further support for the assumption was found in the fact that plants in the extreme classes were less variable in regard to their mean relative height than plants in the intermediate classes.

8. The growth constants of plants ending in the several quartiles were practically identical.

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TABLE I. *Heights of individual plants at successive intervals*

Plant No.	Days											
	7	14	21	28	35	42	49	56	63	70	77	84
	Centimeters											
1	17	33	63	106	148	167	177	177	177	177	177	177
2	16	29	56	87	119	157	200	212	226	226	262	226
3	19	38	69	100	130	172	218	257	277	279	279	279
4	19	36	65	101	139	185	230	231	239	244	244	244
5	21	41	76	117	165	224	281	312	322	325	325	325
6	18	38	77	115	162	206	236	236	241	241	241	241
7	19	34	55	74	105	149	203	229	232	234	234	234
8	21	43	74	108	149	202	258	268	273	273	273	273
9	19	40	73	102	132	166	208	254	292	298	301	301
10	18	39	73	109	143	181	225	271	302	303	303	303
11	20	37	61	88	111	141	178	213	250	264	270	272
12	22	45	84	119	149	185	231	252	264	264	264	264
13	21	41	80	122	153	191	235	281	316	332	332	332
14	19	46	79	111	137	169	198	220	227	228	228	228
15	18	35	71	102	129	160	190	228	257	261	264	264
16	21	44	84	122	156	186	228	275	291	294	300	300
17	18	34	67	102	131	168	206	245	250	265	268	268
18	17	33	63	97	132	182	232	253	266	270	272	272
19	16	35	67	99	128	158	182	210	237	245	249	249
20	18	38	74	105	131	154	170	202	214	217	222	226
21	19	42	75	101	118	140	162	198	230	241	241	241
22	18	38	66	84	111	121	152	187	209	211	213	213
23	19	40	75	105	139	180	227	264	273	277	277	277
24	19	40	75	107	137	175	215	265	310	323	332	339
25	18	35	67	93	122	163	192	186	192	192	192	192
26	17	34	67	99	135	185	243	297	311	315	317	317
27	19	45	89	145	197	220	221	221	221	221	221	221
28	16	30	57	85	124	175	227	269	305	313	313	313
29	18	33	57	82	121	176	233	249	256	256	256	256
30	18	37	72	100	142	197	254	280	281	281	281	281
31	17	36	73	105	143	194	247	294	326	330	330	330
32	15	24	49	74	111	160	208	247	271	280	280	280
33	19	42	79	116	155	203	244	217	281	281	281	281
34	16	35	70	95	125	167	194	214	237	243	246	254
35	18	36	63	78	115	157	190	213	233	238	238	238
37	18	39	65	89	117	155	187	214	240	248	250	252
38	17	37	61	72	93	111	131	150	171	189	189	190
39	21	43	76	95	121	151	170	183	194	198	200	200
41	19	41	73	102	137	178	206	218	223	224	224	224
42	18	33	59	79	106	129	149	166	175	175	175	175
43	19	28	54	84	108	132	153	183	200	211	214	214
44	17	36	66	109	152	191	212	212	212	212	212	212
45	17	35	66	95	120	153	181	197	203	208	208	208
46	18	36	65	86	110	133	158	188	212	219	225	227
47	18	38	66	86	109	137	166	182	187	187	187	187
48	15	31	58	80	111	155	200	218	224	224	224	224
49	17	38	75	109	144	191	236	250	254	254	254	254
50	15	30	68	104	142	198	252	293	318	320	320	320
51	16	32	60	88	126	182	233	260	291	293	293	293
52	17	31	62	103	155	196	203	203	203	203	203	203
53	17	34	60	84	119	163	214	252	264	266	266	266
54	16	36	71	104	144	198	250	288	303	310	310	310
55	16	39	75	96	124	165	204	231	264	278	282	283
56	16	30	62	84	103	121	152	179	200	209	212	212
57	19	38	70	105	144	158	164	164	164	164	164	164
58	16	23	38	57	88	125	157	185	199	202	202	202
59	17	36	70	100	131	169	212	247	278	283	286	286
60	18	37	70	99	134	178	228	231	278	284	295	295

TABLE 2. *Constants for growth and variation in height of Helianthus plants*

Days	Mean Height, Cm.	Increase of Mean Height, Cm.	Standard Deviation	Coefficient of Variability
7	17.93 ± 0.14	7.93 ± 0.14	1.62 ± 1.01	9.03 ± 0.57
14	36.36 ± 0.43	18.43 ± 0.44	4.83 ± 0.30	13.28 ± 0.86
21	67.76 ± 0.78	31.40 ± 0.89	8.93 ± 0.56	13.17 ± 0.84
28	98.10 ± 1.38	30.34 ± 1.59	15.60 ± 1.98	15.90 ± 1.02
35	131.00 ± 1.73	32.90 ± 2.21	19.52 ± 1.22	14.90 ± 0.95
42	169.50 ± 2.21	38.50 ± 2.81	25.00 ± 1.57	14.75 ± 0.94
49	205.50 ± 2.92	36.00 ± 3.67	33.00 ± 2.07	16.06 ± 1.03
56	228.30 ± 3.40	22.80 ± 4.49	38.47 ± 2.41	16.84 ± 1.08
63	247.10 ± 3.80	18.80 ± 5.11	42.92 ± 2.69	17.38 ± 1.12
70	250.50 ± 3.76	3.40 ± 5.35	42.48 ± 2.66	16.95 ± 1.09
77	253.80 ± 3.99	3.30 ± 5.48	45.06 ± 2.82	17.75 ± 1.13
84	254.50 ± 3.89	0.70 ± 5.57	43.90 ± 2.75	17.25 ± 1.11

TABLE 3. *Quartile distribution on each successive day of measurement of plants starting in the first quartile*

	Days											
Quartile	21	28	35	42	49	56	63	70	77	84	Total	Percent of Grand Total
I.	12	9	7	6	5	5	6	6	6	6	68	45.33
II.	2	3	4	3	5	4	3	3	2	2	31	20.67
III.	1	2	2	4	2	3	3	3	4	4	28	18.67
IV.	0	1	2	2	3	3	3	3	3	3	23	15.33
									Grand total 150			
Mean quartile position	1.266	1.666	1.933	2.133	2.200	2.266	2.200	2.200	2.266	2.266		
σ.	.115	.944	1.064	1.089	1.350	1.125	1.170	1.170	1.180	1.180		

TABLE 4. *Quartile distribution on each successive day of measurement of plants starting in the second quartile*

	Days											
Quartile	2	28	35	42	49	56	63	70	77	84	Total	Percent of Grand Total
I.	1	3	2	2	2	3	4	3	3	3	26	18.57
II.	8	6	6	7	5	5	3	4	4	4	52	37.14
III.	5	4	4	2	4	3	4	3	3	3	35	25.00
IV.	0	1	2	3	3	3	3	4	4	4	27	19.29
									Grand total 140			
Mean quartile position	2.285	2.214	2.428	2.428	2.571	2.428	2.428	2.571	2.571	2.571		
σ.	.590	.861	.905	.981	.981	1.051	1.117	1.117	1.117	1.117		

TABLE 5. *Quartile distribution on each successive day of measurement of plants starting in the third quartile*

Days

Quartile	21	28	35	42	49	56	63	70	77	84	Total	Percent of Grand Total
I..........	2	3	4	5	6	5	4	5	4	4	42	28.00
II..........	4	4	4	3	2	2	4	3	5	5	36	24.00
III..........	6	5	4	4	4	6	5	5	4	4	47	31.33
IV..........	3	3	3	3	3	2	2	2	2	2	25	16.67
									Grand total 150			
Mean quartile position......	2.666	2.533	2.400	2.333	2.266	2.333	2.333	2.266	2.66	2.266		
σ............	.945	1.024	1.426	1.136	1.183	1.076	1.012	1.11	.949	.949		

TABLE 6. *Quartile distribution on each successive day of measurement of plants starting in the fourth quartile*

Days

Quartile	21	28	35	42	49	56	63	70	77	84	Total	Percent of Grand Total
I..........	0	0	1	2	2	2	1	1	1	1	11	7.86
II..........	0	1	1	1	1	3	4	4	4	4	23	16.43
III..........	3	4	5	5	7	2	3	4	4	4	41	29.29
IV..........	11	9	7	6	4	7	6	5	5	5	65	46.43
									Grand total 140			
Mean quartile position......	3.785	3.571	3.285	3.071	2.928	3.000	3.000	2.928	2.928	2.928		
σ............	.424	.625	.884	1.034	.963	1.138	1.000	.963	.963	.963		

TABLE 7. *Number and percentage of the measurements falling in the several quartiles for plants starting in a given quartile*

Quartile in which Plants Started	Quartile Positions Observed During Growth of Plants								Total	Theoretical Standard Deviations	Observed Standard Deviations
	I		II		III		IV				
	No.	Percent	No.	Percent	No.	Percent	No.	Percent			
I......	68	45.33	31	20.67	28	18.67	23	15.33	150	3.53	11.92
II......	26	18.57	52	37.14	35	25.00	27	19.29	140	3.64	7.52
III......	42	28.00	36	24.00	47	31.33	25	16.67	150	3.59	4.94
IV......	11	7.86	23	16.43	41	29.29	65	46.43	140	3.63	14.60
Total......	147		142		151		140		580		
Mean percent..		24.94		24.56		26.07		24.43			

TABLE 8. *Quartile distribution on each successive day of measurement of plants ending in the first quartile*

Quartile	14	21	28	35	42	49	56	63	70	77	Total No.	Percent of Grand Total
I.........	6	6	7	7	9	11	12	14	14	15	101	67.33
II.........	3	6	3	3	3	2	3	1	1	0	25	16.67
III.........	4	1	2	0	0	2	0	0	0	0	9	6.00
IV.........	2	2	3	5	3	0	0	0	0	0	15	10.00
Mean quartile position.....	2.133	1.933	2.066	2.200	1.800	1.400	1.200	1.066	1.066	1.000		
σ..............	1.088	.998	1.182	1.327	1.167	.712	.400	.253	.253	0.000		
Grand total ...											150	

TABLE 9. *Quartile distribution on each successive day of measurement of plants ending in second quartile*

Quartile	14	21	28	35	42	49	56	63	70	77	Total No.	Percent of Grand Total
I.........	2	2	3	6	5	3	3	1	1	0	26	18.57
II.........	4	5	4	3	4	8	8	12	12	14	74	52.86
III.........	5	3	4	3	3	1	3	1	1	0	24	17.14
IV.........	3	4	3	2	2	2	0	0	0	0	16	11.43
Mean quartile position...	2.642	2.642	2.500	2.071	2.142	2.142	2.000	2.000	2.000	2.000		
σ...........	.974	1.045	1.500	1.100	1.061	.917	.654	.377	3.77	0.000		
Grand Total...											140	

TABLE 10. *Quartile distribution on each successive day of measurement of plants ending in the third quartile*

Quartile	14	21	28	35	42	49	56	63	70	77	Total No.	Percent of Grand Total
I.........	4	5	4	2	1	1	0	0	0	0	17	11.33
II.........	3	2	3	6	6	4	3	1	1	0	29	19.33
III.........	4	4	5	4	5	5	8	12	14	15	76	50.67
IV.........	4	4	3	3	3	5	4	2	0	0	28	18.67
Mean quartile position.....	2.533	2.466	2.466	2.533	2.666	2.933	3.066	3.066	2.933	3.000		
σ...........	1.148	1.205	1.089	.958	.871	.933	.683	.141	.025	0.000		
Grand total ...											150	

TABLE 11. *Quartile distribution on each successive day of measurement of plants ending in the fourth quartile*

Quartile	14	21	28	35	42	49	56	63	70	77	Total No.	Percent of Grand Total
I.	3	2	1	0	0	0	0	0	0	0	6	4.29
II.	4	1	4	2	1	0	0	0	0	0	12	8.57
III.	2	7	4	8	7	7	4	2	0	0	41	29.29
IV.	5	4	5	4	6	7	10	12	14	14	81	57.86
Mean quartile position. . .	2.642	2.928	2.928	3.142	3.357	3.500	3.714	3.857	4.000	4.000		
σ.	1.137	.965	.965	.643	.611	.500	.455	.353	0.000	0.000		
Grand Total. . .											140	

TABLE 12. *Number and percentage of the measurements falling in the several quartiles for plants ending in a given quartile*

Quartile in which Plants Ended	Quartile Positions Observed During Growth of Plants								Total	Theoretical Standard Deviations	Observed Standard Deviations
	I		II		III		IV				
	No.	Percent	No.	Percent	No.	Percent	No.	Percent			
I.	101	67.33	25	16.67	9	6.00	15	10.00	150	3.55	25.67
II.	26	18.57	74	52.86	24	17.14	16	11.43	140	3.63	16.40
III.	17	11.33	29	19.33	76	50.67	28	18.67	150	3.58	14.36
IV.	6	4.29	12	8.57	41	29.29	81	57.86	140	3.64	20.74
Total.	150		140		150		140		580		
Mean percent. .		22.88		24.36		25.78		24.49			

TABLE 13. *Successive increases in mean height of plants ending in various quartiles.* (*Height in centimeters*)

Increase in mean height of plants since the preceding measurement

Quartile in which Plants Ended.	Days											
	7	14	21	28	35	42	49	56	63	70	77	84
I.	7.8	17.1	29.4	28.8	32.0	26.6	20.3	13.4	8.4	3.5	0.6	0.1
II.	7.8	19.7	31.1	27.5	31.0	37.0	32.5	20.6	14.4	4.1	1.5	1.1
III.	8.2	18.0	31.3	29.8	33.4	43.4	45.7	28.3	15.4	4.6	3.8	0.2
IV	7.9	19.0	34.3	33.2	36.0	45.5	48.7	39.5	29.0	5.7	2.4	0.5

TABLE 14. *Mean quartile position and standard deviation of each plant arranged according to mean quartile classes*

	Mean Quartile Classes										
Pl. No.	1.00–1.75		Pl. No.	1.75–2.50		Pl. No.	2.50–3.25		Pl. No.	3.25–4.00	
	m	σ		*m*	σ		*m*	σ		*m*	σ
1	1.666	1.107	2	1.833	0.688	3	3.000	0.407	5	4.000	0.000
22	1.416	0.760	7	1.750	0.829	4	2.583	0.495	8	3.583	0.496
25	1.583	0.380	11	2.250	1.010	6	3.000	0.912	9	3.416	0.643
38	1.166	0.554	19	1.916	0.281	14	2.750	0.829	10	3.500	0.500
42	1.166	0.554	20	2.000	0.816	15	2.500	0.500	12	3.416	0.497
43	1.166	0.554	21	2.250	0.595	17	2.750	0.433	13	4.000	0.000
45	1.416	0.495	32	2.000	0.912	18	2.583	0.863	16	3.916	0.284
46	1.500	0.500	34	2.000	0.407	26	3.166	0.776	23	3.250	0.433
47	1.333	0.624	35	1.916	0.495	27	2.916	1.189	24	3.750	0.433
48	1.583	0.495	37	2.083	0.495	28	2.666	1.314	30	3.250	0.595
56	1.000	0.000	39	1.916	1.257	29	2.500	0.957	31	3.416	0.162
58	1.000	0.000	44	2.250	1.164	41	2.583	0.641	33	3.750	0.433
			52	1.916	1.116	49	3.166	0.795	50	3.250	1.043
			53	2.333	0.756	51	2.750	1.233	54	3.416	0.956
			57	2.000	1.080	55	2.583	0.760			
						59	3.000	0.707			
						60	3.166	0.556			
Average.....	1.333±.098	.502±.069		2.028±.138	.793±.098		2.804±.129	.786±.091		3.565±.091	.505±.064
No. of Plants.....12			15			17			14		

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Boston

Vol. VI. JULY, 1919 No. 7

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

Smithsonian Institution
AUG 6 1919
National Museum

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PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT 41 NORTH QUEEN STREET, LANCASTER, PA.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879

AMERICAN JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

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The Journal is published monthly, except during August and September. Subscription price, $5.00 a year. Single copies 60 cents, plus postage. Postage will be charged to all foreign countries, except Mexico, Cuba, Porto Rico, Panama, Canal Zone, Republic of Panama, Hawaii, Philippine Islands, Guam, Samoan Islands, and Shanghai. Postage to Canada 20 cents a volume on annual subscriptions; to all other countries in the Postal Union, 40 cents a volume on annual subscriptions.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $5.60 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Fifty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

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Business correspondence, including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or 41 North Queen Street, Lancaster, Pa.

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THE STRUCTURE OF PROTOPLASM[1]

R. A. HARPER

It may seem to many that there has been little progress in recent years in our conceptions as to the constitution of protoplasm. While great advances have been made in our knowledge of the molecular constitution of various proteins *in vitro*, we cannot yet feel sure of the adequacy of the data so obtained as a basis for conceptions as to the molecular constitution of substances found in the living protoplasm. Further, the discovery of such bodies as hormones, vitamines, etc., with their far-reaching physiological significance is after all largely a matter of qualitative observations which have not yet been brought into relation with our knowledge of the visible protoplasmic cell structures. And yet it seems to me that the new data on the chemistry of the colloids and the results of experimental studies in genetics, as well as the more direct observations of cytologists, have been producing some very fundamental changes in our conceptions as to the chemical and physical characteristics of the cell and protoplasm.

I have no new theory of cell organization to propose, but desire to bring together the data from cytology, colloid chemistry, and genetics which bear on this, for all biologists, fundamental problem. That the organization of the cell is the fundamental problem of all biology was never clearer than it is to-day. The theory that all organisms are constituted of cells, each with its own more or less subordinated life history, and that the functions of all organs, tissues, etc., are in the last analysis the functions of cells, has proved the most illuminating, the most universal in its application, of any viewpoint ever developed in the history of biology. It is so familiar, so much a commonplace of all thought and speculation that it seems idle to dwell upon it, and yet it is certainly a unique situation in science that practically the whole developmental series of both plants and animals are *cellular* in organization. A structure so complex as the *cell* must have had an evolutionary history, but the whole evolution of living organisms as we know them is the history of the increasing specialization of structure and corresponding division of labor between cells whose fundamental architecture remains the same.

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[1] Address of the retiring president of the Botanical Society of America, delivered at Pittsburgh, December 31, 1917.

[The **Journal** for June (**6**: 217–272) was issued July 21, 1919.]

The cell theory that higher organisms are complexes of simpler organisms which by their manifold interactions build a more differentiated organic unit was never more firmly established, more thoroughly in harmony with all known facts, than it is to-day. Opponents there are of this doctrine, but even they admit that the great problem to be solved is the discovery of the organization of protoplasm, and this search to discover the real structure of the protoplasm is the goal of all cell studies. There is no real diversity of opinion between biologists as to the significance, the methods, and the goal of cytological investigation. The viewpoint which I wish to emphasize is that the structure of protoplasm is the structure of the cell. The search for some ultra-microscopic structure of living substance as such and more deep-seated than cell structure, has, so far, proved as vain as the older attempts to demonstrate the existence of a vital force.

To the theoretical biologist the biological sciences, old and new, anatomy, surgery, physiology, pharmacology, pathology, bacteriology, and genetics—these great fields of human thought and effort which have borne fruits of such inestimable value for civilization and the progress of the human race are all of interest as they contribute to the explanation of the cell structure and its functions, for to-day no really critical student of biology questions that in the organization of the cell lies the world-old riddle as to the nature of life, its origin, possibilities, and limitations.

The cell theory with its varied implications lies at the center of all biological thought and interest, and the history of the cell theory has been a continual vindication of the methods of observation and analysis as against the loose and easy methods of *a priori* speculation. No philosopher ever guessed that life units as we know them arise only by division, that the fertilization of the egg is the fusion of cells, or that heredity is based on a painstakingly equal division of a germ plasm. Whatever we may believe about pre-established harmonies in the organization of the universe, it is obvious that our minds are not so constituted that it is easy for us to guess the truth or that whatever is true about the cell seems natural and to be expected. The great discoveries of biology have been reached by the laborious and sometimes seemingly almost blind method of trial and error, with error always largely in evidence.

We are inclined to speak of the present as a period of criticism and of the application of fundamental discoveries of science to practical affairs, but it seems to me equally obvious that, as noted, the new data in chemistry as well as from experimental breeding and cytology are gradually and sometimes perhaps almost subconsciously changing our viewpoints as to many of the old conceptions of life and protoplasm. We can perhaps, as I have suggested above, classify in three groups the movements and discoveries which are influencing our conceptions of life structures and life processes. I shall speak of the contributions of cytology, of the chemistry of the colloids, and of genetics to our conceptions of protoplasm.

Data from Cytology

In considering the modern cytological viewpoint as to the structure of the cell, I may first indicate the general sense in which I shall use the terms relating to the cell and its parts. And I must state at once that nothing is further from my intention than to attempt to provide a series of hard and fast definitions of structures as fluctuating and plastic as are the cell and the materials of which it is composed. A word is perhaps necessary here as to the place of definitions in modern science. It is frequently stated that science progresses with the increasing clearness of our definitions, and that much useless controversy would be avoided if contestants were compelled to define their terms. It seems to me that in these statements modern science is accepting and perpetuating a mediaeval dogma which since Darwin has practically lost its significance. In the days of metaphysics and the use of the deductive method in logic, it was of vast importance to know just how much was implied in any term used in a major premise, for on that and that alone depended how much could be gotten out of it in conclusions. Especially when certain conceptions or ideas were assumed to be self-evident or axiomatic, it was vital to know just how much was implied in a given term as self-evident. With modern science, so-called axioms as relating to any matters of scientific importance have largely gone the way of all the earth. With a realization of the supremacy of the inductive method we become less restrictive in our use of terms in our larger interest as to what is really true of the subjects to which they relate. Relatively few modern controversies are due to misunderstanding of each other by the principals. In the dispute between Weismann and Spencer over the inheritance of acquired characters it was no mere matter of terms, and in the flare-up over the meaning of the word *genotype* which followed the visit of Johannsen to this country the obscurity was all in the facts about the constitution of the germ plasm rather than in the various definitions and usages proposed for the term. There is a positive danger in the use of definitions which is obvious in the discussion of the factorial hypothesis raging just now. There is a group of geneticists who would frame a definition of the term *unit factor* so very broad and so empty of all concrete and specific implications that it would become an abstraction like many of the conceptions of mathematics. This is the aim of those who would make Mendelism a system of notation. A mere one factor conjugated with one factor makes two factors, and one factor separated from the other factor gives two independent factors. Representatives of these tendencies sometimes try to win support by accusing their opponents of ignorance that one and one make two and one from two leaves one. No one disputes such mathematical obviousness, but it is the death of science when concrete realities are attenuated to fit such empty generalizations. It is of the very essence of modern scientific method and spirit that it has broken away from such formalism and resists the temptation of the human mind to accept and remain content with such empty formulae. It is a familiar fact to critics of literature and art as well as to scientists that every great advance in knowledge with new concrete discoveries and every great creative age is followed by a period when the new data, instead of being made the basis for further advances, become the shibboleths of new schools. Concepts that have been fruitful and actually represent a great advance in knowledge come to be used in a mystical, absolute, and all-embracing sense quite unimplied in their original usage.

We may return from this brief disquisition on definitions and terminology to our theme with an illustration of this misuse of terms. The term *protoplasm* as used by Purkinje was merely descriptive and concrete in its reference to the material of young embryos. Von Mohl made it more definite and still more specific as the term applied to the slimy viscid material which makes up the content of the cell inside the cell wall, which latter structure to him was still a very important if not essential element in his conception of the cell. He was also still far from free of mystical conceptions as to the nature of life and life-processes as something quite outside of and beyond the properties of protoplasm. Huxley made protoplasm the physical basis of life, a conception which still allowed for much play of the imagination as to the existence of forces and principles which could be imagined, as Bergson imagined them, to use the protoplasm as the mere physical substratum for the manifestation of their autonomous activities. In the elimination from modern biology of mystical conceptions as to vital force or forces in a super-physical realm we have come, however, to transfer a certain element of mysticism to the conception *protoplasm* itself, and to refer to the properties of protoplasm in quite the same fashion as the mediaevalists did to vital force, formative principles, etc. We say or obviously imply too easily, especially to our elementary students, that all the functions of life in plants and animals are explainable as due to the properties of protoplasm to assimilate, grow, divide, etc. Such statements are true so far as we know, but they are so general, so empty of all concrete specification as to the facts of assimilation, growth, and reproduction, that they are mere formalisms quite as deadening in the end to the real progress of research and discovery as were the conceptions of the "vital spirits" of mediaeval physiology in their relations to the functions of nerves, muscles, etc., or the nutrient, reproductive, sentient, etc. soul powers of Aristotle—conceptions which when accepted as finalities tended to discourage rather than to stimulate research. In stating, then, that I shall use the term *protoplasm* as referring broadly to the whole sum of materials which make up the cell, including the cell wall, metaplasm, starch, fat, cell sap, even inorganic crystals and water of inclusion, nothing is farther from my intention than to give a definition of protoplasm. Protoplasm is in most intimate relations of interchange with its environment, and, further, there is surely no hard and fast line between its external environment and its so-called internal environment, using the latter term to refer, for example, to foods and wastes dissolved in the vacuolar cell sap.

With this understanding as to definitions, I shall use the word *cell* as referring to the whole protoplasmic life unit, including the wall or envelopes of every kind. This will include in the cell conception multinucleated coenocytes and plasmodia as well, though it is obvious that here the content of the term is stretched until its usefulness becomes impaired. Still, I may note without discussion that this, in my opinion, is much better than to regard coenocytes and plasmodia as non-cellular or as tissues with cell walls omitted, as many do. We have here a case of which there are so many, where nature simply mocks our categories and shows the weakness of attempts to separate by definition objects that are so intimately connected in their evolutionary history.

If, as I have suggested above, the visible organization, its architecture, is the structure of the protoplasm, we are confronted with the further significant consideration, which the numerous studies on the algae, fungi, and all the invertebrate phyla which have appeared in the last two decades have brought out very clearly, that this organization of the cell is everywhere broadly speaking the same. From the one-celled alga or fungus to the highest plant or animal, the differentiation of nucleus, cytoplasm, chromosomes, spindle fibers, etc., is everywhere present, and in their general nature and functions and in their essential interrelations these structures are the same. I am not overlooking the fact that nuclei have not been satisfactorily proved to exist in the bacteria, or that the central body of the blue green algae can perhaps best be considered as only a very aberrant form of nucleus. The macro- and micro-nuclei of the infusoria also imply a quite different type of organization from that of the common uninucleated cell. We must not, however, allow ourselves to be blinded by these facts, significant as they are and fruitful as they may become in deepening our conceptions of cell organization, to the great outstanding fact that evolution as we know it has not consisted in the production of new types of protoplasmic structure or cellular organization, but in the development of constantly greater specialization and division of labor between larger and larger groups of cells. The obviousness of this fact is brought out most conspicuously by the diagrams of a typical cell. Whether taken from the text-books of botany or zoology, these schematic figures all show a striking agreement as to the more fundamental structural features of a so-called typical cell. The differences between plants and animals, or between Pleurococcus and the pine, are not indicated primarily in the general organization of their cells.

We shall see most clearly the difference between the former and the new viewpoints when we note the disappearance of the old corpuscular theories of protoplasmic structure from the literature of present-day cytology. To explain certain evidences of the transmission of acquired characters

Darwin thought it legitimate to postulate particles, the "gemmules," which carry such characters. Spencer thought all functions of the organism must be represented by "physiological units" of the protoplasm. Haeckel explained assimilation as the "perigenesis of plastidules." Weismann brought this method of attack to its climax in his complex series of assumed biophores, determinants, ids, and idants; though perhaps Heidenhain's graded series of life units, including super- as well as subcellular grades, has outdone that of Weismann. The cytologist has been unable to discover in the viscous semi-liquid, semi-solid colloidal mass of the cell any adequate evidence of the existence of such a particulate structure.

What seems to me the most important advance in our knowledge of cell architecture has been in the direction of the recognition of localized spatially differentiated regions of the cell body in which certain processes occur. To illustrate this point we may take the case of our increasing knowledge of the elaioplasts. Bodies associated with the appearance of oil in the cells have long been known in certain tissues of Funkia and Ornithogalum. Wakker's work on the elaioplasts of Vanilla vastly increased the clearness of our conceptions as to these structures. In the cells of the vanilla plant the elaioplasts appear as quite definitely bounded, specifically organized regions in the cytoplasm. They show a reticulated stroma with more or less included oil which with specific treatments may be made to exude upon their surfaces. But they are not sharply bounded, and they are quite irregular in outline. In general they are not so liquid or so differentiated from their surroundings as to round up, and here again we have the evidence as in the case of the chloroplasts that the cell elements may be sufficiently viscous to maintain irregular outlines by virtue of their semi-solid condition. Beer has been able to recognize and has figured elaioplasts in many tissues in the Compositae.

Other scattering observations indicate that they are of widespread occurrence in connection with oil production in many families of plants, and yet no one would deny that fatty materials may appear in cells, especially in the liverworts and fungi and in animals, in the entire absence of any specially differentiated region of the cytoplasm for their formation. We get thus, as I conceive, a clear-cut notion of the modern cytologist's conception of protoplasmic structure. Oil may be formed anywhere in the cell. If the conditions are favorable for its production in large amounts, this production is likely to become localized and to bring with it marked regional transformations, so that we can even speak of such a region as an elaioplast or organ of the cell. If such conditions are permanent the organ is permanent, and by virtue of the extreme non-diffusibility of colloids and their tendency to form surface tension membranes, may be perpetuated even through long periods of inactivity.

Similarly the plastids are regions of the cell body in which carbohydrates are deposited in solid or semi-solid form as starch grains, to be later redissolved and transported throughout the cell and the tissue or organ and to more or less distant parts of the plant. That the plastid is to be regarded as a region of the protoplasmic complex rather than as a differentiated and definitely delimited body is shown with especial clearness in the case of those algae whose chloroplasts are of irregularly lobed or frayed-out outlines. The functions of the chloroplast in forming assimilation starch are strictly dependent on the presence in it of green chlorophyl, and cytologically the chloroplast is perhaps little more than an area of the cytoplasm impregnated or infiltrated with chlorophyl. The leucoplast is obviously less differentiated than the chloroplast. That in the marginal lobes of the chloroplast the green pigmentation seems frequently to pass over by an imperceptible shading off in color into the gray of the adjacent cytoplasm is an observation easy to make on many of these algae. Hydrodictyon is especially well suited to illustrate the relations of the starch-bearing region to the remainder of the protoplasmic mass. The earlier writers had attempted to recognize a zonal differentiation in the Hydrodictyon cell, and the inner green zone or plastid with its pyrenoids was described as being free from nuclei; but Timberlake's sections show that pyrenoids and nuclei are scattered through the whole thickness of the primordial utricle about the central vacuole. Presumptively at least the whole primordial utricle is infiltrated with chlorophyl in the mature cell. The appearance of the cells of the young and growing nets of Hydrodictyon is also very suggestive as to the nature of plastids and their delimitation. As my photographs show, and as has been noted before, the ends of the young cells are gray and free of green chlorophyl, the latter occupying a band-shaped and very vaguely delimited zone in the middle region of the cell. As the nets grow larger, however, the chlorophyl spreads toward the ends of the cells until they are uniformly green in color, the number of pyrenoids increases from one to several, and the appearances suggest not the growth of a specific body but the increase of materials concerned with starch formation, pyrenoids and chlorophyl, and their gradual spread throughout the whole primordial utricle. This spread and increase of the pyrenoids involves their division, but the chlorophyl apparently merely diffuses out into the adjacent colorless regions of the cytoplasm as it increases in amount. In *Botrydium*, for example, and in the higher plants the chloroplasts appear as quite sharply delimited bodies of definite form and outline which themselves, at least at certain stages, arise by division, though their transmission as such through the egg and pollen tube may still be regarded as in question. It has not been adequately shown in any case, however, that the plastid has a specific membrane of its own like the plasma membrane or that of the nucleus. It is, so far as the microscope shows, a green-pigmented, denser region of the cytoplasm—a group of elements of the polyphase colloidal cytoplasmic system. The absence of a specific membrane about the plastid is shown especially by the widely recognized occurrence of stroma starch.

Starch formation in the plant has been studied chemically much more than cytologically, and our various theories of light assimilation have been based very largely on the chemical study of chlorophyl and observations on the appearance and disappearance of starch grains in their relation to the income and outgo of CO_2 and O_2 in the cell or leaf. Timberlake's studies of the relation of the pyrenoids to starch formation in Hydrodictyon, in which he shows that each grain begins as a segment of a pyrenoid which undergoes a series of microscopically visible changes in becoming a starch grain, indicate that starch formation in these cases at least involves physical transformations of masses which can be studied with the microscope as the atomic molecular readjustments with which the chemist deals. The work of Lutman on Closterium and of McAllister on Anthoceros have added a further series of observations in the field of the cytology of metabolism which indicate that the microscope is to play an increasingly important rôle in the study of these problems which have hitherto seemed open only to the methods of chemical analysis. It may be of interest to note that while the most widely current chemical theories of light assimilation seem to favor a katalytic theory of carbohydrate formation, all these cytological data point just as clearly to the acceptance of a metabolic theory. What is known cytologically of leucoplasts and chromoplasts agrees equally well with the conception of the plastid as a region of the protoplasm specialized with reference to carbohydrate metabolism. The leucoplast without its starch grain or grains is, as noted above, apparently a very slightly differentiated body indeed. The loss of chlorophyl has left it with very little to distinguish it from the adjacent cytoplasm, and yet the layer about a large stratified grain of storage starch is the seat of highly characteristic chemical transformations which, as Denniston's studies show, involve the formation of a cytologically demonstrable differentiated zone between the plastid and the stratified portion of the grain.

If Schimper is correct that the chromoplasts are derived from the other plastids by chemical transformations, we have in the frequently crystalline appearance of the latter the evidence that here again the plastid in the older tissue cells is a region in which the deposit of pigment crystals takes place.

The carbohydrate-forming plastids have gained a greater degree of permanency in the cell than the elaioplasts, so far as we know them now, and it is of the greatest significance that, associated with the growth and division of the cell, they so regularly arise by division. This division is a simple constriction both in the case of the plastids and of the pyrenoids. It is clear that pyrenoids may arise *de novo* in the cytoplasm. Timberlake could not convince himself that they certainly persist through swarmspore formation in Hydrodictyon, and Gilbert Smith has recently shown that they certainly arise *de novo* in three of the four cells produced by the double division of a mother cell in Scenedesmus and certain other algae. As to the chloroplasts, as noted, Schimper's evidence of their persistence through the egg stage, though his figures are clear enough for Daphne and Hyacinthus, cannot be regarded to-day as completely convincing in spite of the *quasi* support his theories have received from the theory of the mitochondrial origin of all plastids. In such forms as Hydrodictyon the division of the chlorophyl-bearing plasm is the division of the primordial utricle itself, but in the division of the tissue cells of the higher plants the independent constriction and bipartition of the plastid as such is to be reckoned with as a cytological fact of the first significance, and we must simply confess that we know nothing of the fundamental physical-chemical processes by which such constrictions are initiated and carried through. Our increasing knowledge of the chemistry of the carbohydrate and protein molecules has given us no clue to the solution of the problem of division, whether in the case of the simple bipartitions of these plastids or the complex mechanical processes involved in karyo- and cytokinesis. Under such circumstances, however, it is worse than useless to use such phrases as "tendency of protoplasm to divide" or "tendency of living materials to reproduce by bipartition." The problem is to be attacked, as it seems to me, from the standpoint of the newer evidence and conceptions as to the physical conditions existing in polyphase and compound colloidal systems rather than from that of a study of the chemical organization of the protein molecule.

Our knowledge of the visible structures concerned with oil and carbohydrate formation in the cell, as well as our knowledge of the sources of the materials and the possible chemical stages in the synthesis of starch from CO_2 and H_2O, give us a standpoint from which to obtain suggestions as to the organization of protoplasm and indicate the progressive localization of these processes in the protoplasmic mass with the formation of more and more definitely differentiated organs which finally reproduce by division.

In sharpest contrast with these contributions of cytology to our knowledge of oil and carbohydrate formation in the cells, I would set much of the modern literature of the chondriosomes, mitochondria, etc. Here, it seems to me, instead of a critical study of protoplasmic structure in relation to cell functions, we have in too many cases a mere reactionary movement taking its origin in the old view that we should hope to find in the cell the physical embodiments of the gemmules, pangens, ids, plastidules, etc., of a generation past. In so far as Meves, Duesberg, and others have endeavored to associate the formation of muscle rods, nerve fibrillae, etc., with visible elements of the embryonic cytoplasm, their work is, of course, highly suggestive and stimulating. I do not wish now to attempt to pronounce a judgment on the evidence as given by the authors noted above as to the relation of so-called chondriosomes, mitochondria, etc., to the processes of cellular differentiation in animal tissues. Their figures are in some degree at least convincing, and if confirmed will, in my opinion, mark one of the longest forward steps that have yet been taken in our investigation of the physical basis of life and life processes. Much less satisfactory is the evidence of Regaud, Dubreuil, and others who attempt to associate the chondriosomes with fat-formation, cell secretion, etc. If muscle rods, nerve fibrillae, etc., are actually formed out of unit semi-solid elements already existing in the cytoplasm of the embryonic cells, it certainly strengthens greatly what we may call a physical-mechanical, as contrasted with a chemical, viewpoint as to life processes. The visible structure of the protoplasm is not then, as Lundegårdh would say, the expression of the chemical transformations going on at the moment, but something vastly more permanent and mechanical. It should be regarded as a physical system of relatively permanent unit elements in definite space relations to each other and undergoing more or less progressive and cyclic rearrangements with resulting new and specialized capacities and functions. No more necessary investigations lie before us in cytology than those bearing on the question as to the histogenesis of such complex tissues as muscle and nerve fibers.

In the case of the attempts of Guilliermond and others to trace plastids, anthocyan vacuoles, metachromatic granules, pyrenoids, and other structures of the adult differentiated plant cell to cytoplasmic chondriosomes of the embryonic cells, the situation is clearer, if not so promising of illuminating new results. In a word, it seems to me that none of the evidence so far adduced as to a specific genetic relationship between chondriosomes and plant plastids is in any way adequate. It is not only that there is something like an equivalence in the weight of testimony in the literature on both sides of the question, but none of the evidence either pro or con seems to me to rest on adequately checked-up and convincing data of observation. That granules, rods, strands, etc., can be observed in the cytoplasm is undoubted and has long been known. The claim that those taking a given stain after a given fixation can all be classed together as coördinate unit elements, while suggestive, needs much further confirmatory proof like that which has been accumulated for the individuality and permanence of the chromosomes. That in certain cells the plastids can be recognized as very small cytoplasmic bodies with no starch in them was adequately established by Schimper, but that the plastid bodies necessarily and regularly arise from the chondriosomes it seems to me is by no means proved by such crude and diagrammatic figures and seriations as those so far presented.

In the present situation a somewhat sweeping criticism is, it seems to me, justified. I think we must all agree that the bulk of the literature of the plant chondriosome is a mere tabulation of the appearance of variously fixed and colored particles in the cell body with the hope that such bodies may later be found to be specific and fundamentally significant. I cannot attach great importance to the contention that such bodies, as a result of their locations in the cell, are distributed with approximate equality to the two daughter cells in division. The significance of such a distribution depends entirely on the specificity of the bodies and their relations to cell functions.

I have illustrated my viewpoint as to protoplasmic structure rather fully by the cases of the elaioplasts, plastids, and chondriosomes. The recent literature on these structures seems to me especially suggestive of the tendencies which are promising and of those which are reactionary in present-day cytological literature.

The conceptions of cytologists as to the chromosomes as units of cell structure are much more definitely fixed as a result of the vastly greater amount of attention which has been devoted to them. It is of interest to note that cytologists have devoted their attention almost entirely to the phenomena of reproduction and heredity. The phenomena of direct and indirect nuclear and cell division in asexual reproduction on the one hand, and of chromosome reduction and nuclear and cell fusion in connection with sexual reproduction on the other, have furnished such a wealth of easily accessible data that the processes of cell metabolism, growth, and irritability have been relatively neglected.

In the same way those physiologists who have used principally the methods of chemical analysis and physical measurements have found just these problems which the cytologist has neglected the more accessible. There has grown up thus an interesting division of the field according to the methods of study used by the investigator into cellular physiology or cytology, dealing largely with reproduction, and what is generally known as plant physiology, dealing quite as exclusively with metabolism, growth, and irritability. This might seem to indicate that the physiology of reproduction is a negligible field. At least one recent text-book of plant physiology states that the subject of reproduction has been adequately treated under the head of morphology. Such vagaries of viewpoint and opinion may safely be left to the future for correction, but it is the conspicuous fact that cytologists have largely concentrated their attention upon the phenomena of reproduction with the result that unquestionably the chromosomes are the best known bodies of their size in the whole field of science. The attempt to question the validity of the evidence for the existence of these structures in the living cell and to class them and other cell structures as products of fixation, staining, etc., has broken down completely. The older studies of Strasburger on division figures in living cells have been confirmed and extended by Lundegårdh and others. The essential characteristics of the chromosomes as to their form, size, and position in the cell and with reference to each other are recognizable in cells that are still alive and going through the processes of division. Their persistence in the resting condition of the cell in many plants either quite unchanged or as the so-called prochromosomes has been adequately demonstrated in fixed and stained material by Rosenberg, Overton, and others. Leaving aside the doubtful cases of the bacteria, blue green algae, and perhaps some protozoa, it is well nigh universally agreed that every cell has its specific complement of chromosomes quite definite in number, size, and perhaps in relative position in the cell body.

Further, they reproduce only by the division of parent chromosomes—a division which again outside the cases of certain protozoans gives evidence of its painstakingly equational character. We have then in the chromosomes subsidiary bodies in the protoplasm which duplicate in their permanence and method of reproduction the cell itself. As Boveri has emphasized, each chromosome has a life history of its own. It divides for reproduction, and each daughter chromosome grows to the size of the parent chromosome and in cyclic fashion is again divided. This is the basis for the doctrine of the individuality of the chromosome and has been used in support of the much vaguer, panmeristic doctrines of cell division and of the older theories that the cell is a complex of lesser life units, each, however, endowed with the full complement of the essential cell characters—the capacity to assimilate, grow, and divide cyclically into equivalent daughter units. These latter conclusions, however, it seems to me far outrun the facts. The data from elaioplasts, plastids, vacuoles, nucleoles, etc., suggest that, while permanence and reproduction by division can be present in various degrees in all these structures, they are rather acquired conditions worked out and developed in connection with the metabolism of the cell as a whole than essential characteristics of life units which by their combination then make the assimilation, growth, and division of the cell as a whole possible.

The permanence and equational division of the chromosomes are none the less the great outstanding discoveries of cytology as to cell organization, and here, as in the case of the constriction of the plastids, the underlying physical and chemical phenomena involved have so far escaped discovery. Neither chemistry nor physics furnish any data which aid the cytologist to discover why the segments of the spireme thread should split longitudinally or why the spireme should divide transversely to form the chromosomes. For the separation of the daughter chromosomes we have the possibility of contractility in the spindle fibers, protoplasmic streaming even, etc.—processes with adequate physical analogies—but the initial splitting of the chromosomes before the spindle is formed is quite without parallel in the behavior of atoms, molecules, or larger colloidal particles as known to the chemist and physicist. The division of plastids, chromosomes, and, as we shall note presently, centrosomes, as well as the pairing of chromosomes in cell and nuclear fusion, are basic data of cytology that so far differentiate cellular organization and processes from those of unorganized matter.

As to the functions of the chromosomes, we have a mass of evidence, which has been so many times and so well summarized that I need not rehearse it here, that they in some way provide for the so-called transmission of the hereditary characters. The evidence that the chromosomes are a physical basis for heredity is, it seems to me, entirely convincing and adequate. It is particularly significant that with the intensive study of heredity which began with the Mendelian revival nothing has been discovered which in any essential way invalidates the evidence from cytology that at least certain hereditary characters are in some way transmitted through the chromosomes. Indeed, the parallelism between the processes involved in chromosome reduction as described by cytologists and the theoretically postulated behavior of factors in segregation is regarded as one of the strong points in favor of the whole Mendelian theory.

On the other hand, when we touch the question as to just how the chromosomes function in heredity, and more definitely how the qualities of the tissues and organs and of the organism as a whole which are borne by them come to expression in the morphogenesis both of the embryo and the adult, we find ourselves again at an *impasse*. It is easy to say that the serration of a leaf edge is an inherited character transmitted by the chromosomes of any parent plant which possesses it, and the statement harmonizes with practically all known data of cytology and experimental genetics, but to attempt to give this generalized formula of concepts concrete reality by telling how the serration is represented in the chromosome and how it comes to expression in the many-celled leaf is quite beyond us as yet. It is quite possible that we are astray in our conception of representation on the one hand and the characters of many-celled organs on the other. At any rate, the method of functioning of the chromosomes in heredity is a problem of the future, though we can have no doubt that they are just as definitely related to hereditary transmission, whatever that implies, as are the plastids to carbohydrate metabolism.

From the standpoint of our question as to the structure of protoplasm, I think we may say that the chromosomes are each regions or portions of the protoplasm which by reason of the localization and specialization of certain functions and processes in them have come in some degree as has the cell itself to have a permanent unity and identity, and to arise only by division of parent chromosomes. The protoplasm is not an aggregate of such bodies, but its activities have been specialized and localized till such bodies as chromosomes have resulted. As a polyphase colloidal system it has furnished the internal condition for the development of the greater and greater differentiation, specificity, and fixity of its phases.

The centrosome or central body with its obvious relation to cell dynamics in the division both of nucleus and cytoplasm, and with its more recently discovered relation to cell movements as the blepharoplast, with its frequently, if not always, minute size and resemblance to other granules or groups of granules in the protoplasm, has been a main support for all theories of cell structure involving the idea of living granules of ultramicroscopic or at least sub-cellular size. Heidenhain makes it the type of one of his grades of life units. The evidence that the granules (centrioles) or groups of granules (microcentra) regularly arise by division of parent granules or groups in nuclear division in animals and lower plants is convincing, and yet in Bryophytes, Pteridophytes, and Cycads whose nuclei divide without the presence of centrosomes they appear apparently *de novo* as blepharoplasts in the formation of the motile male cells and even in the last few preceding nuclear divisions. Such evidence as this is most convincing, as it seems to me, as to the close interrelations of structure and function in protoplasmic organization. I have developed elsewhere the idea of the central body as a region of connection between nucleus and cytoplasm and for the formation of fibrillar kinoplasm in connection with my studies of free cell formation in the ascus. The data as to the behavior of the central body in the mildews may be taken as embodying the idea of a cell organ which I am here presenting. The central body is a permanent structure in the mildew cell. I have been able to trace it at all stages of their growth and development both sexual and asexual. The central bodies arise only by division and when the nuclei fuse the central bodies fuse, and yet it seems to me this is no ground for regarding them as life units, individuals, in the full sense in which the cell is such a unit; much less for regarding the cell structure as an aggregate of such living units. The position and relation of the central body to the nucleus and cytoplasm in free cell formation give the best evidence as to its nature. It is, as noted, a region of the cell at which the chromatin of the nucleus and the cytoplasm come into specific relations by contact and where fibrillar kinoplasm is formed and passes out to form the plasma membrane of the young daughter cell, the ascospore. That a granule of some specific chemical compound could play such a rôle it seems to me is by no means so easy an assumption as that in this particular region where the nucleus and cytoplasm are so definitely connected we have a concentration, a localization, of formative processes which results in the production of the disc-like central body and the radiating fibrils which ultimately form the plasma membrane of the ascospore. The activity of the processes dies down after cell division is complete but increases again when a spindle is to be formed for the next nuclear division.

From this point of view it is quite conceivable that, as in the ferns and cycads, a central body should appear *de novo* at the poles of the spindles in the androgones, where fibrillar kinoplasm aggregates, and persist later as the region from which the fibrillar cilia arise in the metamorphosis of the androcyte into the motile antherozoid.

I have avoided introducing in this connection the still too vague conceptions of intracellular enzymes and their role in cell activities; but the processes of free cell formation in the ascus can certainly be well conceived as involving a fermentative katalytic action of the contents of the nucleus on the adjacent cytoplasm in the region of the central body which results in the diffusion outward from the center of the material of the radiating fibrils.

The central body in its obvious dynamic relations to the other structures of the cell illustrates especially well the conception of cell structure which implies differentiated regions of a colloidal system in which special processes have become localized and tend to remain fixed. I have endeavored in my discussion of the conceptions of the cytologist as to cell structure to present them as far as possible from the standpoint of our present conceptions of the protoplasm as a polyphase colloidal system or complex of such systems. We may turn now to the more specific discussion of the influence which the new discoveries in the chemistry of the colloidal condition has had on our conception of cell structure.

Data from the Chemistry of the Colloidal Condition

The contributions arising from the modern study of matter in the colloidal condition to our conceptions of protoplasm have been, in my opinion, of the most far-reaching significance. We can perhaps appreciate most fully the change in the relations of biology and chemistry which has come with the development of the chemistry of the colloids if we consider the contributions of chemistry to that oldest and most elusive of biological problems—the nature and origin of adaptive form. It is here perhaps in the problems of morphogenesis that chemistry has appeared most helpless and the biologist has felt most justified in resorting to mystical and vitalistic conceptions of regulative principles, developmental tendencies, etc. I have referred above to our inability to conceive how the chromosomes as bearers of the hereditary characters can control the development of the inherited form characters in ontogeny.

The attempt to explain the form of organisms on the basis of analogies with crystalline forms and configurations has failed so conspicuously that biologists might well perhaps feel justified in questioning the possibility of a chemical theory of plant and animal form. A glance at the older and the more recent attempts in this direction is perhaps of interest.

Grew in 1672 felt the necessity of attacking the problem of the causation of plant forms from the standpoint of the forms of the crystals of salts found in plant juices. Out of the few and simple crystal types which he could isolate he put together groups which were supposed to explain the development of the trunk, the divergence of branches from the main axis at various angles, the serration of leaf margins, the formation of spiral vessels, and, to Grew most important of all, the very method by which the "fibers" are spun together to form the cell walls in Dame Nature's endless weaving of the lace-like patterns of the plant tissues. This was in 1672 and dates the beginning of the cell theory. In 1903 Przibram, undeterred by the failures of three hundred years, again claims to lay the foundations of a true theory of morphogenesis by a comparison of form development in both plants and animals with the growth, twinning, regeneration, etc., of crystals. He compares the replacement of a broken-off angle of a crystal to the regeneration of a salamander's leg and the replacement of a leaf tip. The bifurcation of a fern frond looks to him like the twinning of a crystal.

Przibram's figures and ideas are perhaps not as crude as those of Grew, but the general consensus of biological opinion is, undoubtedly, that no progress has been made along these lines. Such viewpoints lead simply into a hopeless *cul de sac* and seem to make mockery of the whole attempt at a chemical explanation of life phenomena.

The change that came with the recognition of the colloidal condition as a phase of material existence comparable in significance to the crystalline condition, the solution condition, the gaseous condition, etc., was, as I have said, of the most far-reaching significance to the biologist. The formation of permanent or semi-permanent suspensions and the demonstration that conditions of equilibrium could be reached in such systems followed by the recognition and careful descriptive characterization of sols and gels as studied *in vitro* brought chemistry and cytology on a common ground. One of the most important results of the study of the colloidal condition is the recognition of the fact that the units in colloidal systems, especially those of proteids, carbohydrates, etc., are large enough to be distinguishable at least with our present microscopes.

With the recognition that semi-fluid systems in physical-chemical equilibrium can be constituted of units larger than the molecules and within range of study at least with the ultra microscope, the possibility of assuming a complex meta-microscopic organization of the protoplasm in which the essentials of vital processes are carried on is made less plausible. These foam and emulsion structures are the first and most obvious characteristics of protoplasm which the microscope reveals. Even more, the evidence that in this colloidal condition the transition from liquid to solid, from sol to gel, tends especially to pass into an indefinite series of gradations gave a basis for the explanation of that mixture of the properties of solids and liquids which has puzzled students of protoplasm. In the light of the effects of temperature changes on the phases of a colloidal system the familiar biological phenomena of heat rigor, *rigor mortis*, protoplasmic coagulation, etc., could be at least paralleled by phenomena *in vitro*. The reversibility of the processes in certain cases and their irreversibility in others also parallel other familiar cytological data.

The biologist could only demur when the chemist demanded that he describe the phenomena of plant form in terms of crystallography and the processes of nuclear and cell division in terms of the chemical reactions of substances in solution. But Rhumbler has made real progress in describing the form of the shells of Foraminifera in terms of surface tensions in an anomogenous semi-liquid system, that is, a system whose liquidity varies in its different parts so that the homologous surface tension angles are always equal though non-homologous angles may be widely different. To say that the chromosomes go into solution in the telophases and reappear as crystals in the prophases was palpably absurd, but to describe the change as the passage of a gel into the continuous phase of a sol and its reverse is a chemical equivalent for many descriptions of the breaking up of the chromosomes in the telophases and their reconstitution in the prophases as found in current cytological literature.

The concrete data of the chemistry of the colloidal state so far relate chiefly to simple two-phase systems, and the conditions of the two phases are conceived as a more and a less watery phase of a single compound, though there is nothing in the conception of a sol to suggest such a limitation. Little has been done, too, toward the analysis of those intermediate conditions between the sol and the gel, though it is perhaps just here that the bulk of cytological phenomena belong. Still, there are those who on the meager data already available have formulated theories of protoplasmic structure in terms of colloidal systems.

To present a picture of the present chemical theories of protoplasm we must recognize two quite divergent tendencies or schools of thought which are largely represented amongst biologists and chemists of the day. First, the group of which we may take Verworn as a representative, who hold that a single very complex chemical compound, for Verworn the biogen, built up on the benzol ring and with Ehrlich side chains for dissociation and restitution, is the essential physical basis of life. The other visible constituents of the cell are to be regarded as more or less accessory. Life is the dissociation and restitution of biogens; all else is secondary.

The second school, which we may represent by Hofmeister, holds that the protoplasm is essentially an aggregate of compounds of varying complexity. Hofmeister takes at once the conception of a polyphase colloidal system as the basis of his account of cell organization.

The theory of a single living substance passes easily into the conception of specific chemical substances for each species of organism as developed by Kossel and carried still further by Correns in his theory of self- and cross-sterility and -fertility as due to similarity and dissimilarity of the specific individual substances in the same or different individuals. Correns has developed the interesting criticism of the doctrine of individual stuffs that even if the possible number of stereoisomers of a carbohydrate with 40 carbon atoms in its molecule was as great as 2^{40}, about a billion stereoisomers, there still would not be enough to provide a different one for each rye plant, for example, since a single crop of rye in Europe amounts to 41 billion individual rye plants. Reichert would have the genus, species, variety, race, sex, individual, and even tissue or organ stuffs the countless stereoisomers which the complexity of the protein molecule makes possible.

The theories which postulate a single living substance have been developed by many into a doctrine of protoplasmic structure as a simple, even a two-phased system. Bütschli's foam or alveolar theory would find the essential substance in the continuous phase while the contents of the alveolae, the disperse phase, is simply a watery cell sap. Various granular inclusions in the continuous phase are also assumed. Beijerinck and

Lepeschkin and all adherents of the so-called granular theories would make the disperse phase, consisting of the discrete particles, the bioplasts of Altmann, the plasomes of Wiesner, the chondra of Rohde, and, in general, the microsomes of older authors, the real living material units, while they would hold the continuous phase, their hyaloplasm or interstitial jelly, as dead or unorganized, perhaps a secretion of the granules. If Bütschli's theory is correct, the protoplasm would be a foam. If Beijerinck's and Lepeschkin's theory is right it would perhaps be better called an emulsion, using the terms to distinguish the relative importance of the disperse and continuous phases.

The crude simplicity and general inadequacy of these latter conceptions and the bitterness of the controversies which have been waged over them, especially by Bütschli and his followers, have done much to bring the whole subject of protoplasmic organization into disrepute. On the other hand the conception of protoplasm as an aggregate of complex compounds, a polyphase colloidal system or system of systems, seems to do much more adequate justice to the observed facts. The sols as at present commonly described permit only a single continuous phase, though the discontinuous phase might consist of an indefinite number of discrete bodies homogeneously distributed or even with zonation or other localization of certain elements according to their chemical and physical interrelations. But substances of greater viscosity may separate out as reticula and give us thus several continuous or partially continuous phases. The existence of such interlacing strands and films is a familiar fact of protoplasmic structure in the killed and fixed condition, and can also be observed in living pollen mother cells, spores, etc. Frommann's familiar figures of the protoplasm in the end cells of glandular hairs suggest such structures.

Hofmeister emphasizes the importance of the surface tension membranes between the different substances in the cell which are due to their immiscibility as furnishing just the means necessary to hinder diffusion between the different regions of the cell and to make possible the maintenance of the various structures and organs of the cell which the microscope reveals. This immiscibility of the different assumed substances makes possible also the different functions of the various cell parts, functions, as he points out, involving the simultaneous occurrence in different parts of the cell of different and frequently opposite chemical transformations such as hydration and dehydration, oxidation and reduction, synthesis and decomposition. The decomposition of glycocoll to urea presupposes a specific seriation of the reactions involved which implies an independent activity of the various intermediary substances produced such as could not exist if the protoplasm were a homogeneous mixture. The possibility of such reactions presupposes, for Hofmeister, a so-called chemical organization of the cell. The endless series of ferments and their products are thus separated and enabled to carry out their independent acceleratory reactions by virtue of the inter-faces formed between them as phases of a colloidal system. The slowness of the diffusion of colloidal ferments leads to their becoming rooted as it were in certain regions of the cell most favorable for their activity.

No one can deny that this picture of the chemical organization of the cell does full justice to many of the observed facts of cytology as well as to the processes of physiological chemistry. The conception of the enzyme is perhaps over-emphasized in view of the slight knowledge which we have of the chemical nature of these elusive compounds. But the localization of functions in the cell and the special acceleration of certain general reactions in particular regions of the cell are conceptions which can be connected directly with the cytology of starch formation, oil formation, the building of astral rays, spindle fibres, and cilia, the production of cell pigments, etc. Hofmeister does not at all emphasize the phase relations of these various constituents of the cell, and it is obvious that a polyphase colloidal system does not of itself involve such structural differentiations as Hofmeister assumes and the microscope reveals. No matter how great the number of phases or how intimate their interrelations are assumed to be, the polyphase system in itself might be in general homogeneous throughout its whole extent, that is, the particular phases and their interrelations might repeat themselves in all directions through the mass in a uniform and undifferentiated sequence. That is, just as in a two-phase system any one unit area large enough to include the two phases will be essentially like any other unit area of equal size in the mass, so in the polyphase system the mass is naturally conceived as including an indefinite number of repetitions of unit areas essentially alike as to their makeup. The polyphase system is merely the two-phase system increased as to the number of its constituent elements. It is only by adding the conceptions of zonation and other types of segregation of the elements of the polyphase system due to their chemical interrelations that we arrive at a parallelism with what is seen in the cell.

We can put this in another way which will at once bring out the peculiarities of cell organization as compared with polyphase systems *in vitro* by noting that the polyphase system may be homogeneous in the respect that it shows no axial differentiation. Whatever the interrelations of the phases as to internal and external, sol or gel, they will be the same at the top as at the bottom, in the front as at the rear, on the right side as on the left side. The only exception is that any polyphase system will differ on its surface as compared with its interior, that is, will show some concentric zonation. This difference has been exploited to the full in the numerous recent attempts to utilize the data of colloid chemistry in the analysis of the structure and functions of the plasma membrane. It would take us too far afield to review the data in this complicated subject further than to note that none of the theories—lipoid, mosaic, adsorption, filter, etc., have contributed anything very positive to the general theories of protoplasmic structure.

Aside from this concentricity there is no evidence of symmetry relations or polarity in a polyphase colloidal system as such. On the other hand, polarity is one of the most obviously demonstrable characteristics of cell organization. Polarity in herbaceous and woody shoots is known to all. That the polarity of the shoot as a whole is due to the polarity of the individual cells has been made highly probable by appropriate experiments. That polarity is also a property of cells of simpler plants and doubtless of all cells is shown by Tobler's elaborate and far-reaching demonstrations for algal cells.

The simplest possible statement of the facts as to polarity in the cell brings out at once the conspicuous differences between cell organization and that of a polyphase colloidal system as such. Greil, who is perhaps the latest to attack the problem from a theoretic standpoint, in his labored effort to bring all morphogenetic factors into an epigenetic rubric can get no further with the polarity of the animal egg than to say that it would be very remarkable if with such long-continued and mighty growth of the yolk-containing egg cell an entirely homogeneous consistency, tectonic, of the cell organization should be maintained. Polarity is for Greil simply a matter of eccentricity in the deposit of accumulated reserve materials, and yet this simple statement of its eccentricity at once differentiates the telolecithal yolk-bearing egg from a polyphase colloidal system as such. Greil quite ignores the fact that Tobler has shown the existence of pronounced polarity in algal cells which contain little or no reserve foodstuffs and that it has never been possible satisfactorily to associate the polarity of shoots, etc., of the higher plants with any special distribution of either "formative" or reserve stuffs.

Greil goes so far as to say that the egg furnishes only bilateral polarity as a form-determining factor in the development of the embryo. This standpoint implies a program rather than present achievement, but it is interesting to us as showing what stress a confirmed epigeneticist like Greil lays on polarity as a characteristic of cell organization. To say that the egg furnishes only bilateral polarity as a form-determining factor implies that it will be possible to show how the bilateral polarity of the different species of eggs varies, since many eggs show bilateral polarity and yet with similar environment the product of their development is very different.

It is not, of course, shown to be impossible that polarity may be in its essence simply the expression of the two-sided or bipolar distribution of the visible structural elements of the cell such as the nucleus, centrosome, plastids, etc. It would seem *a priori* more probable that the eccentricity in the deposit of such metaplasmic inclusions as yolk, starch, fat, aleurone, etc., is the expression of a polarity in the more fundamental architecture of the cell rather than its cause. This is the sort of polarity shown in Rabl's classic figure which represents in my opinion the most adequate diagram of cell organization so far conceived. The swarmspores of an alga like Pediastrum with their plastids, mouth-piece, blepharoplasts, cilia, etc., are visibly highly polarized structures, and it will be of great interest to determine whether this visible polarity coincides with the major axes of the adult cell of Pediastrum and the apparent affinities which determine the orientation of each cell with reference to its fellows and to the form of the colony as a whole. This problem is by no means insoluble with the modern culture methods available for keeping such algae in large numbers under continuous observation.

That in Pediastrum the swarmspores show polarity in at least two axes and that this polarity determines their mutual interactions and final position in the colony is shown conclusively, as I have pointed out elsewhere, by the symmetrical organization of the colony and the method of its formation from a group of freely swimming cell units. Polarity exists in probably all the tissue cells of metaphytes and coenobic plants and in many protophytes. It is apparently independent of the uni- or multinucleated condition of the cell, which shows that it is in some cases at least a more generalized characteristic of the cell as a whole rather than a mere expression of the space relations of the nucleus and cytoplasm in a diagram like that of Rabl. Bilateral and radial symmetry are shown also in the cells of desmids, diatoms, and other protophytes, these latter showing in Rhumbler's term the anomogeneity of the protoplasm as a liquid. It is sufficiently clear, it seems to me, that in the presence of polarity and the various symmetry relations we have a fundamental distinction between cell organization and that of polyphase colloidal systems as they are commonly produced *in vitro*.

Furthermore, the outstanding fact not sufficiently recognized in the theory of colloids as at present developed is that we may have at least more or less differentiated colloidal systems within a colloidal system. The cell must at least be conceived as a complex of such colloidal systems, some possibly simple two-phase systems as perhaps a vacuolated nucleole, some polyphase as the nucleus taken as a whole within the cytoplasm. In such cases the interior system as a whole will show a tendency to form convex surfaces toward the enveloping system or systems. Perhaps the nearest approach to an experimental demonstration of such an organization is found in Hardy's development of the evidence for secondary interior phases. If a mixture of gelatine and water is cooled to a certain temperature, we obtain a watery interior phase in a continuum of denser gelatin; if now the cooling is continued small particles appear in the watery disperse phase which may make chains which anastomose to form reticula constituting a secondary interior gelatine-rich phase. In this case we should still have no polarity in the mass as a whole. The interior phases would be repeated equally in all directions throughout the whole system. None the less the experiments as described are highly suggestive as to the physical relations under which the internal structures of such a complex system as the nucleus exist. Such secondary interior phases doubtless exist in the case of the granular precipitates in vacuoles and the frequently observed inclusions in the nucleoli. They also provide for the development of filar and reticulated structures in colloidal systems in which the general consistency is still quite fluid. What appears perhaps most inadequate in the data of colloidal chemistry as a basis for the conceptions developed from a study of the cell itself is the failure of students of unorganized colloids to emphasize the evidence for the existence of the thready reticulated and filar structures which are so familiar to the cytologist in fixed material.

Boresch has also described filar structures in the living protoplasm of moss cells whose method of occurrence, sensitiveness to environment, reagents, etc., suggest their resemblance to the reticulated chains of granules produced by Hardy. As Lundegårdh has pointed out, these figures of Boresch show many resemblances to the so-called myelin structures and are doubtless in some cases the same cell elements which have been variously described as mitochondria, *Chondriokonten*, etc. They are far less thread-like than the highly differentiated, clean-cut fibers of the asters, spindles, etc., of our fixed preparations, but are interesting as showing that thready structures as well as granules, foams, emulsions, etc., are to be reckoned with as constituents of protoplasm. These fibers of Boresch, as also the mitochondria, show no polar orientation except perhaps as they are more or less passively influenced by the karyokinetic figures in dividing cells. Their form cannot hence be ascribed to the operation of fields of force, magnetic polarities, etc., operating on the cell substances, as is so frequently claimed for the fibers of the karyokinetic figures.

To provide an adequate basis for understanding the observed facts of polarity, however, it seems to me that the conception of compound aggregate polyphase systems is more suggestive than these attempted analogies between the magnetic poles and their fields of force and the karyokinetic figures. The suspension of one or several polyphase systems within another polyphase system is entirely harmonious with what we know of the high viscosity of many of the constituents of protoplasm. In the spatial arrangement and interrelations of these systems polar differences of the most diversified types are bound to arise in the mass as a whole and express themselves in the form and relative rigidity and surface tension of different parts, as well as in the interrelations between the cells of a group in contact.

But the most important factor which the cytologist must recognize with reference to the development of such systems as are shown in the organization of the cell is the time element. In contrast with any known unorganized system, the cell propagates itself by division, as Weismann has so adequately and fully emphasized. This means that the protoplasmic structure is not formed *de novo* in reproduction, but has perpetuated itself as such from the remote geologic periods when life first appeared upon the earth. The spatial interrelations of the colloidal elements and systems of the cell are not, then, such as we might expect to duplicate by mixture *in vitro*. They are in some degree at least the product of the infinitely varied external and internal environmental changes to which the cell in its long evolutionary history has been exposed.

Genetics and the Structure of Protoplasm

It is perhaps unfair to the geneticists to consider the great contributions which they have made in recent years in the light of their bearing on current theories of protoplasmic structure. The adherents of the doctrine of unit factors assert repeatedly that they are quite unwilling to commit themselves as to the form in which these unit factors are to be conceived as existing in the germ plasm. We are told that they are merely presenting facts as to the behavior of visible characters in breeding experiments and that we may imagine any sort of representation of these factors in the germ plasm which we please. And yet views so widely held and so stimulating of research as the factorial hypotheses are certain to influence strongly at least our *a priori* conceptions of the structure of the germ plasm and of protoplasm in general.

The serial arrangement of such factors in the chromosomes seems to be involved in that parallelism between the data as to chromosome reduction and the segregation of factors which has afforded such strong support for the whole Mendelian theory. We may note in passing that Trow points out regarding the evidence of serial arrangement derived from linkage that the numerical data used as the basis of the assumption of a serial order of the factors constitute "a type of representation common to every set of phenomena which can be expressed as percentages."

Still, it may be noted further that if we are not to regard the chromosomes as so many chains of factorial beads there is no other hypothesis which has any recognized standing at present with either cytologists or geneticists. The development of the conception of the cell as a polyphase colloidal system seems to point in another direction, but it has led to no very definite ideas as to the way in which the characters of the adult many-celled organism are represented in the germ plasm. It is doubtless true, as is so strongly felt by the newer epigeneticists like Greil, that the idea of a representation of characters of a many-celled organism, especially those due to the inter-relations of the cells, in the chromosomes cloaks a vast amount of obscurity. These latter biogenetic characters must certainly be put in a different category from the characters which Detto has called *metidentical* and whose inheritance offers relatively little theoretic difficulty.

It is certainly worth while to consider most carefully these conceptions based on the facts of breeding from the viewpoint of their influence on theories of protoplasmic structure. The day is past for the explanation of vital phenomena by the assumption of units or particles endowed with properties which explain their assigned functions. Nowhere is the need of a critical consciousness of the whole protoplasm question shown more clearly than in the frequent looseness of such assumptions.

The assumption is that we can draw specific conclusions as to the constitution of the germ plasm from the behavior of visible characters in the multicellular organism, even though this behavior is so complex that it necessitates the assumption of several independent units in the germ plasm for one apparently individual character. Bateson's amoeba would have to be represented as of gigantic size in order to get points of origin for all the characters that have come out of it and been eliminated in evolution. There can be no question that with the intensive study of the past two decades the problems of heredity have been found to be vastly more complex than it was hoped they would appear when treated by the Mendelian method of analysis. The pangens of De Vries, at least in the earlier form of his theory, were regarded as relatively few in number and broadly pervasive in their visible effects in the organism. We are now apparently more inclined to Weismann's conception of indefinitely numerous determiners in more or less fixed space relations to each other.

The realization of the weakness of Mendelism in relation to facts as to the all-pervasiveness and interdependence of plant characters has led some of the defenders of the theory to assert that each unit factor may possibly influence every part of the mature plant.

The difficulty with all such assumptions of hereditary units of whatever kind is more fundamental. Take the case of the serrations of the leaf of the common nettle. Correns told us in 1903 that entire margins and serrate margins were due to a factor in the germ plasm for serratures paired with one for entire margin, and the whole was made an example of dominance and segregation. It is to be noted that Correns found teeth weakly developed in his recessives, and while so far as I am aware no one since has gone over the matter I am willing to predict on the basis of my studies on sugar and starch characters and aleurone color in corn that a whole series of intermediates between serrate and entire can be found and that a present day student instead of saying that there is one factor for toothed margin would say there are several or perhaps even twenty.

If serrateness and entirety were found to be absolutely hard and fast categories, units in behavior, there might be something in favor of assuming as a working hypothesis that each was represented by an equally hard and fast, definitely limited section of a chromosome. But if there are all degrees of variation from entire to deeply serrate, the existence of a series of units in the germ plasm, one for each depth of serrateness, is not obviously suggested. The series of fluctuating variants has a unity to the human mind quite as natural as any one of the particular grades of serrateness. This is evidently felt vaguely by those who assume modifying factors and factors of fluctuating potency. To assume, however, that we have explained anything or in any way contributed to clear up our knowledge of germ plasm or heredity by saying that the fluctuating behavior of the visible characters is explained by modifying factors in the germ plasm is a preformationism which would have put even Bonnet to the blush. My point is here that at every step the possibilities of the structure of the germ plasm as well as the visible behavior of characters must be kept in mind.

Perhaps the most obvious weakness of all these theories of units of protoplasmic structure and unit factors in heredity is that they carry in them the vices of the old preformationism. They seem too much like attempts to explain visible and familiar complexity by the assumption of a parallel complexity in the germ plasm, and this in spite of the generally admitted *incommensurability* of cell organization and metaphyte organization. Driesch, with all his tendency to mysticism, must be credited with having recognized and made clear that the facts of nuclear and cell division and the resulting perpetuation of the hereditary complex make it impossible to assume a spatial configuration of the germ plasm in three dimensions parallel to that of the many-celled organism as a whole. The theory of multiple unit factors attempts to maintain in lesser degree this same parallelism—with the added difficulty that the germ plasm which is to be equationally divided must even contain a number of unit factors for each character of the organism.

It is of interest to note in connection with these hypotheses of particulate structure in the protoplasm the available data as to the size of the various elements involved.

Human blood corpuscles	7,500	micro μ	
Anthrax bacillus	4,000–5,000	micro μ	
Cocci	500–1,000	micro μ	
Chromosome:			
Primula Kewensis with 18 chromosomes (Farmer)	1,262 × 1,110	micro μ	Vol. .8141 cubic μ
Primula Kewensis with 36 chromosomes (Farmer)	1,022 × 874	micro μ	Vol. .4088 cubic μ
Chromosome from macromere of Crepidula (Conklin)			Vol. 5.2 cubic μ
Chromosome from micromere of Crepidula (Conklin)			Vol. 2.6 cubic μ
Granules of central body (Marquette mss.)	300–500	micro μ	
Smallest gold particles observed in hydrosols	6–15	micro μ	
The same in non-permanent suspensions	75–200	micro μ	
Molecule of soluble starch (Lobry de Bruyn)	5.	micro μ	
Haemoglobin molecule	2.5	micro μ	
Casein molecule	2.4	micro μ	
Chloroform molecule (Jäger)	0.8	micro μ	
Alcohol molecule	0.5	micro μ	
Hydrogen molecule (O. E. Meyer)	0.1	micro μ	

The granules of the central body which relate themselves so conspicuously to spindle formation are perhaps of the same order of magnitude as some very small chromosomes, and a Micrococcus with all the characters necessary for growth, life history, and reproduction is again of about the same size.

Our positive knowledge of the size of both protein and carbohydrate molecules is very limited, but the figures given indicate that for example the linear dimensions of the chromosomes of the ordinary form of *Primula Kewensis* are some hundreds of times those of the molecules of starch and haemoglobin. There is ample space in these chromosomes for a number of molecules equal to the most extreme demands of the factorial hypotheses if each factor can be represented by a single molecule or even a group of molecules. These particular chromosomes too are by no means large. I have chosen them as illustrations because Farmer and Conklin respectively seem to have measured them with unusual care.

It is sometimes suggested that a factor may be embodied in a single molecule or by a mass of a single compound. It seems probable that colors and similar metidentical characters are due to single compounds or mixtures of a few compounds in the cells of the metaphyte body. That however the complex of cellular interactions including the regulation of the order and relative number of a whole series of cell divisions such as are involved in producing the serratures on the margin of a leaf can be represented by a molecule or group of molecules in a chromosome is hard to conceive. It would seem more natural to regard such organic regulations as the expression of the capacities for interaction of the complex cell mechanism as a living unit in its entirety. The delicately balanced and adjusted 1–5–10 relation between the cells of a bilaterally symmetrical sixteen-celled colony of *Pediastrum Boryanum* seem to be achieved by the interactions of the swarmspores acting as independent units each with a definite polarized organization and capacity to respond to delicate contact and pressure stimuli.

In any case we need more evidence as to the size of protein molecules before comparisons of the size of molecules and chromosomes can have much significance. Of more importance at present is the well-established evidence of specific mass relations between the various parts of the cell unit as a whole considered as a polyphase system, though here again the significance of the facts in their relation to the problem of protoplasmic organization is not yet clear. Strasburger had shown in 1893 that there is a tendency in young meristematic plant cells to the maintenance of a constant volume relation between the nucleus and the cytoplasm, the ratio of nuclear diameter to cell diameter being something like 2:3.

Gerassimow in his classic discoveries of methods for producing and culturing binucleated cells in Spirogyra and other members of the Conjugatae established experimentally the existence of a nucleo-cytoplasmic relation of mass in these forms, as have R. Hertwig and Boveri for various animal types. Gerassimow's data, however, do not cover the problems of chromosome size and number, though it is safe enough to assume that his binucleated cells of Spirogyra contain twice the number of chromosomes found in the uninucleated cells.

Conklin finds that the actual size of the chromosomes in the macromeres and micromeres of Crepidula varies with the size of the nucleus in which they lie but in lesser degree. "The average volume of the chromosomes from the larger nuclei is 5.2 cubic μ and of those from the small nuclei about 2.6 cubic μ. While the volumes of the nuclei as a whole are to each other as about 5:1, the volumes of their individual chromosomes are to each other as 2:1. There is a tendency to constancy in chromosome size, and yet, "just as the size of the nucleus is connected with the volume of the cytoplasm in which it lies, so the size of the chromosomes is connected with the volume of the nucleus from which they come." There is a tendency to a constant chromosome-nucleus relation of mass just as there is a nucleo-cytoplasmic relation of mass.

If in embryological and later development there were a gradual reduction in the size of the nuclei, a corresponding reduction in the size of the chromosomes might be taken as evidence of a distribution of tissue determiners in the crude Weismannian sense, but while, as Conklin shows, there is no nuclear growth of 100 percent after each division there is a growth of five to nine per cent in the volume of the nucleus with each division during early cleavage and a growth of one percent during later cleavage.

Of course these early divisions studied by Conklin and others relate to the development of the general symmetry relations and the *Anlagen* of systems and organs of the mature organism rather than to the production of the definitive *Anlagen* for the tissues. For plants at least the totipotence of the cells of the adult is evidence against the use of mass relations in the germ plasm at different stages of ontogeny as proof of its corpuscular constitution.

Meek's rather crude conception of a definite width increase in the chromosomes as we pass from the lower to more highly specialized organisms has apparently been refuted by Farmer's more careful measurements and analysis. Farmer finds that in *Primula Kewensis* the size of the nucleus varies with the number of chromosomes which go to make it up rather than with the total mass of the chromatic material. As quoted in the table above, the form of *Primula Kewensis* with double the chromosome number of its parents has larger nuclei but the total mass of its chromatin is the same. The doubling of the chromosome number has been brought about by dividing each chromosome of the parent species into two equal chromosomes. The size of the nucleus is in some degree influenced by the number of chromosomes rather than by the mass of chromatin as such. As Boveri holds, the surface area of the chromosomes rather than their total mass appears to be significant in influencing the nucleo-cytoplasmic mass relation.

The measurements of the mass of the germ plasm at various stages of development of the individual so far as they have been carried do however show clearly some tendency to the maintenance of more or less definite mass relations between chromosomes, nucleus, and cytoplasm—that is to the maintenance of a sort of mass equilibrium between the parts of the polyphase cell system. The data as given would indicate a higher degree of mass constancy in the chromosomes than in the nucleus as a whole and in the nucleus than in the cell as a whole.

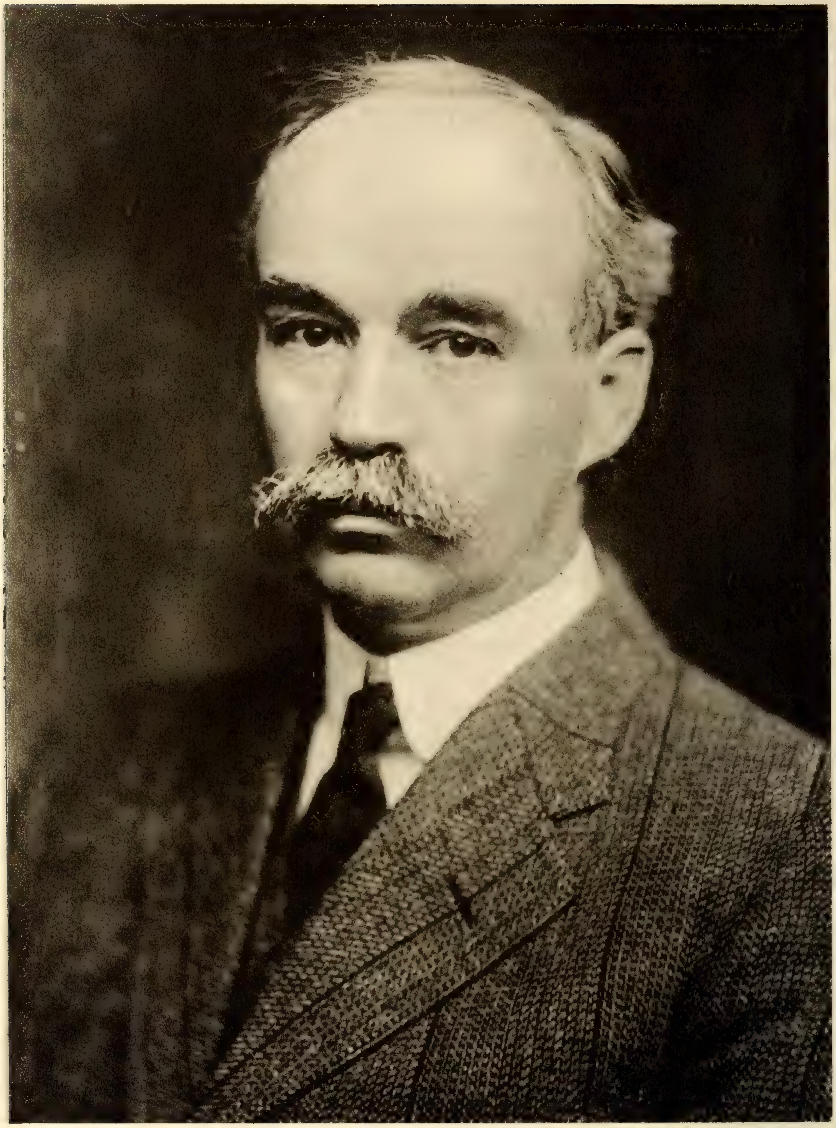
The rather cataclysmic features of the early embryonic periods of development do not obscure entirely the tendency to constancy in the organization of the cell as a whole. Such elements as cell polarity based on the space relations of nucleus, centrosome, and cytoplasmic mass, the relative shape and position of special masses of food reserves, etc., all tend to remain constant or to change by slow progressive transformations and modifications.

In eggs and macrospores overloaded with large masses of yolk or starch as temporary food reserves, the obvious tendency is to regain the balance between nucleus and cytoplasm normal for the cells of the given species in its adult form. A wider range of data in this whole field of the size relations of the various cell constituents is needed as a basis for the further development of the conception of the cell as a complex of colloidal systems.

Farmer's results on two series of fern varieties are not consistent as to the relation of chromosome number to cell size, though for the lady fern series Strasburger's ratio that the diameter of the nucleus is to that of the cell as 2:3 holds good. As Farmer notes, however, the small size and large number of chromosomes in the ferns make them unfavorable material for such studies.

The evidence is certainly clear that the polarity and the mass relations of the parts of the cell, chromosomes to nucleus, and nucleus to cytoplasm, are in some degree specific, as is also the tendency to return to the norm for these relations when they have been disturbed. The further development and refinement of these concepts is of much importance for our conceptions of cell organization and for the transition from the old viewpoint that protoplasm as a substance has a specific structure to the conception that the fundamental organization of living material is expressed in the structure of the cell.

The attempts to recognize plasmodia, coenocytes, syncytia, etc., as protoplasmic rather than cellular are, it seems to me, superficial and misleading. The old attempts to solve the problem of protoplasmic behavior by the assumption that it is composed of physiological units, biophores, determiners, plasomes, pangens, etc., and the newer conception that its essential elements are unit factors, are, it seems to me, being merged in the conception that the structure of protoplasm is the structure of the cell as an organized system and itself the unit in all the complex interactions by which the egg develops into the specialized and differentiated many-celled organism.



Geo. F. Atkinson

GEORGE FRANCIS ATKINSON[1]

W. G. Farlow, Roland Thaxter, and L. H. Bailey

Professor George Francis Atkinson died November 14, 1918, in a hospital at Tacoma, Washington, of pneumonia resulting from influenza. He had been on the Coast since September, collecting fungi. It is thought that he contracted influenza more readily from exposure and overwork, as he found the collecting unusually attractive and worked long hours with great energy.

Professor Atkinson was a botanist of wide reputation, connected for the latter part of his life with Cornell University. In June, 1917, he was relieved of teaching and administrative work at Cornell, continuing on a research professorship until the regular period of retirement should have been reached. Early in the season of 1918 he took the field in the eastern and southern states, expecting to complete the year in extensive collecting in the Pacific Coast region. He was devoting himself to a monograph of fleshy fungi, expecting to publish in several volumes.

In recognition of his mycological and other work, he was elected to membership in the National Academy of Sciences at the spring meeting of 1918. He was an associate editor of the Botanical Gazette, fellow of the American Association for the Advancement of Science, member of the American Philosophical Society and of the national botanical societies, having been president of the Botanical Society of America 1907 to 1909. He was a member of Phi Beta Kappa and Sigma Xi.

He was born January 26, 1854, at Raisinville, Michigan, and entered Olivet College in 1878, later going to Cornell where he received a degree with the class of 1885. He was assistant and later associate professor of entomology and zoology at the University of North Carolina, going there in 1885. He took the chair of botany and zoology and became botanist of the Agricultural Experiment Station at the University of South Carolina; in 1889 he became professor of biology, and biologist at the Experiment Station, in the Alabama Polytechnic Institute. In 1892 he was called to Cornell as assistant professor of cryptogamic botany, becoming associate professor in 1893, and head of the department of botany in 1896 in succession to Professor A. N. Prentiss.

At Cornell he was eminently successful as a teacher of advanced students, of whom a large number are now scattered over the world.

Professor Atkinson was versatile as well as industrious and energetic, and his numerous botanical writings cover a large field. Besides his educational treatises, he published a number of papers on cytological, morphological, physiological, and phylogenetic subjects. His main work, however, was devoted to the systematic study of cryptogams, especially of fungi. In 1890 he published in the Annals of Botany a "Monograph of the Lemaneaceae of the United States," of which he had in preparation a revision. This monograph and a "Note on *Entothrix grande* Wolle," published in the Botanical Gazette (1892), were his only papers on algae.

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[1] Prepared at the request of the council of the Botanical Society of America.

While stationed in the Carolinas and in Alabama, his work naturally was concerned with the local species, for the most part those which attack important crops, especially cotton, and for some years after he removed to Cornell he prepared bulletins, issued by the Experiment Station, on injurious fungi, of which Bulletin No. 73 (1894) and No. 94 (1895) were among the most elaborate.

But even while in Alabama and the Carolinas, his preference for purely systematic studies was manifest, as shown by his papers on Cercospora and Ravenelia from Alabama, Erysipheae from Carolina and Alabama and, in connection with von Schrenk, on "Some Fungi of Blowing Rock, North Carolina."

The "Studies and Illustrations of Mushrooms Edible and Poisonous," Cornell Bulletins 138 and 168 (1897, 1899) were the forerunners of the book "Mushrooms Edible and Poisonous" (1901), with many excellent photographic illustrations made by the author, the work through which Atkinson is best known to the general public. In recent years Atkinson had limited his work more and more to a minute study of the development of the carpophores of the higher Agaricaceae and to a systematic revision of certain genera. He was devoting himself to the preparation of an extensive illustrated work on the "Fleshy Fungi of North America," for which he was exceptionally well equipped. While in the South he was able to gain a practical knowledge of the interesting fungi of this region, and at Cornell was in a position to familiarize himself by frequent excursions with the very rich and varied mycologic flora of the environs of Ithaca. Through his personal acquaintance with Peck he was able to acquire a detailed knowledge of the fungi of New York such as could be obtained only by word of mouth. He had also been able to visit the most important mycological herbaria of Europe, and had made it a rule to botanize in regions where well-known mycologists had worked, that he might see living specimens of the species described in their publications and thus be better able to compare them with species known to him in America.

It is greatly to be regretted that the important work for which he was so well equipped, and for which he had accumulated such extensive material, including several thousand remarkable photographs, should have been brought suddenly to an end when, from his age and robust health, he had every reason to look forward to its successful completion.

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Compiled by Harry M. Fitzpatrick

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VIABILITY OF DETACHED ROOT-CAP CELLS

L. Knudson

One can find in various texts the statement that the root-cap cells of plants die and are sloughed off, and it is probably the general opinion among botanists that the root-cap cells are either dead when they are sloughed off or that they die soon thereafter. Thus, in Jost's Plant Physiology (translation by Gibson, p. 283) the statements concerning the root-cap read as follows: "Its cells are short-lived, but they are constantly being renewed. In spite of this renewal the root-cap does not increase in size because the older cells die off in front and are cast off as new ones are formed."

That the root-cap cells, when sloughed off, are not necessarily dead or short-lived but may persist for many days, seems to be substantiated by various observations made by the writer with a number of different plants. In view of the increasing attention being devoted to the subject of root excretions, it seems desirable to make record of these incidental observations.

The observations here recorded were made primarily on the root-cap cells of corn, although similar observations were made with Canada field pea. The plants were grown in water cultures under sterile conditions, that is, with the roots growing in the entire absence of microorganisms. Pfeffer's nutrient solution was used, with the replacement of dibasic potassium phosphate for monobasic potassium phosphate and with or without one half percent sucrose.

The root-cap cells generally collected at the bottom of the culture vessel, appearing as slimy masses, the amount increasing with the age of the culture and the cells always being more abundant in the sucrose cultures. The cells were sometimes isolated, sometimes in chains of from two to seven cells, again in plates, and occasionally entire root-caps were noted. These cells were always in a healthful condition. They were well filled with protoplasm and each possessed a conspicuous nucleus centrally placed.

That the sloughed-off cells are not short-lived is borne out by the following: Various corn cultures, both with and without sugar, were examined. When the cultures were forty-five days old, the precipitates in the culture vessels were examined and in no case could a dead cell be found. Every cell was well supplied with protoplasm, and that the cells were living was apparent not only from their general appearance but also from the fact that the cells could be plasmolyzed by a glycerine solution and recovery followed.

Since in these experiments seedlings were transplanted to the culture vessels at the outset, and since some of the root-cap cells were sloughed off immediately, it seems fair to conclude that at the time they were examined some of the cells might have been forty-five days old.

In the case of Canada field pea cultures similar results were noted, and even more striking results were obtained as a result of an experiment. In two sucrose cultures (one half percent sucrose) with Canada field pea, all detached root-cap cells were found alive at the end of fifty days. To test further the viability of the cells, the plants were removed from two of the sucrose culture solutions and the solutions were left exposed to the air. Various moulds and yeasts developed in the culture solutions, but despite the contaminations the detached root-cap cells were still alive at the end of twenty-one days more.

Some of these root-cap cells must have been sloughed off during the first days of the experiment, and therefore must have maintained themselves alive, after becoming detached, for a period of seventy-one days. It was necessary to conclude the experiment in order to examine all the cells, so that the maximum period of viability could not be determined.

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Vol. VI. OCTOBER, 1919 No. 8

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

Smithsonian Institution
NOV 25 1919
National Museum

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PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
At 41 North Queen Street, Lancaster, Pa.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania,
under the act of March 3, 1879

AMERICAN
JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

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The Journal is published monthly, except during August and September. Subscription price, $5.00 a year. Single copies 60 cents, plus postage. Postage will be charged to all foreign countries, except Mexico, Cuba, Porto Rico, Panama, Canal Zone, Republic of Panama, Hawaii, Philippine Islands, Guam, Samoan Islands, and Shanghai. Postage to Canada 20 cents a volume on annual subscriptions; to all other countries in the Postal Union, 40 cents a volume on annual subscriptions.

The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

Papers are limited to 20 pages in length, exclusive of plates, except that additional pages may be arranged for at cost rate to authors (approximately $3.00 a page).

Proofs should be corrected immediately on receipt, and returned to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y.

Illustrations, other than zinc etchings, and tabular matter should, in general, be restricted to approximately ten percent of the number of pages of text. Authors may be charged at cost rates for illustrations and tabular matter in excess of that amount (approximately $5.60 a page for tabular matter; 15 cents per square inch for half-tones).

Separates should be ordered when proof is returned. Fifty copies without cover will be supplied free; cover and additional copies at cost.

Remittances should be made payable to American Journal of Botany. Ten cents must be added to all checks not drawn on New York City banks.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Correspondence concerning editorial matters should be addressed to Prof. C. E. Allen, University of Wisconsin, Madison, Wisconsin.

Business correspondence, including notice of change of address, and directions concerning reprints, should be addressed to American Journal of Botany, Brooklyn Botanic Garden, Brooklyn, N. Y., or 41 North Queen Street, Lancaster, Pa.

NOV 26 1919
National Museum

AMERICAN
JOURNAL OF BOTANY

Vol. VI October, 1919 No. 8

VENATION AND SENESCENCE OF POLYEMBRYONIC CITRUS PLANTS

M. R. Ensign

The evidence for senescence in plants has until recently been *nil.* Benedict (1915) has, however, brought forth a considerable amount of data indicating that the venation in leaves of woody plants is greatly influenced by the age of the plant. As the plant passes from youth to old age, the time being reckoned since its origin from seed, the areas of leaf tissue enclosed by the smallest branches of the fibrovascular bundles become relatively smaller. These he terms vein-islets. In other words, the proportion of fibrovascular tissue in the leaves increases with the age of the plant. Propagation by cuttings, furthermore, does not alter at all the venation in the leaf. It is only when reproduction is by seed that a juvenile venation is obtained.

In view of this fact it seems desirable to ascertain whether or not a seedling produced apogamously shows any signs of senescence as compared with a seedling produced from a fertilized egg. Fortunately we have in various species of Citrus a phenomenon which makes these plants desirable for such an investigation. A single seed may produce more than one seedling—a condition known as polyembryony. One of the embryos is produced as a result of sexual fusion, the additional embryo or embryos by budding of the nucellus. Therefore if any differences are found in the seedlings or plants produced from a single seed, some evidence would be available indicative of the significance of sexual and apogamous reproduction in rejuvenation processes.

Polyembryony

The term polyembryony means, of course, the presence of many embryos in a seed. This condition is not altogether unusual, yet the great majority of seeds produce upon germination but a single plant, *i.e.*, they are not polyembryonic.

Strasburger (1878), who made an extensive and detailed study of this phenomenon from the cytological and morphological standpoint, names the following plants which exhibit polyembryony: *Santalum album*, *Sinningia*

[The **Journal** for July (**6**: 273–310) was issued Aug. 16, 1919.]

lindleyana, Nothoscordum fragrans, Citrus species, *Mangifera indica, Evonymus latifolius, Coelebogyne ilicifolia, Epheara* sp., *Cheiranthus cheiri, Vicium album,* and certain orchids. Coulter, Barnes, and Cowles (1910) state that polyembryony is much more common among gymnosperms than among angiosperms.

The most important question connected with polyembryony has to do with the origin of those plants produced in excess of the normal number from a single seed. When seeds normally produce but a single embryo, this is usually considered to arise from the fertilized egg within the embryo sac, being, therefore, gametic. In Citrus species, according to Strasburger (1878), one plant arises in this manner, while the others arise from the nucellar tissue. The latter, therefore, are apogamous. In other species, variations from this type of polyembryony are described. For example, in *Santalum album* and in *Cypripedium Caleolis* there are two fertilized eggs which develop. But in Citrus species, no other origin than that already noted has ever been found. This, however, does not mean that, when Citrus seeds produce only one plant, that one is always of gametic origin. In fact, Strasburger makes it clear that there is no way of distinguishing the gametic from the apogamous seedlings at the time of their germination except by sectioning. Sometimes one and sometimes the other emerges first, and in other cases only the apogamous plant develops. Osawa (1912) confirms these observations.

In some cases a marked difference in the size of the polyembryonic seedlings is noted. This question is deferred for discussion later.

METHODS AND MATERIALS

Plants. The seeds, from which the polyembryonic seedlings were grown for this study, were taken from grape fruit secured at the local market. These seeds were planted, one in each pot, and allowed to grow in the greenhouse. The first lot were planted by Dr. Knudson and were two years old at the time this study was undertaken. All of the determinations on the venation character were made from leaves of this lot. A second lot was planted later from which other determinations on relative size of plants, etc., were made.

That similar environmental conditions surrounded the polyembryonic seedlings which were to be compared is quite well insured, since both the plants from a given seed germinate at the same time and develop under identical conditions so far as factors which may be controlled are concerned. One factor which undoubtedly plays an important part in determining the relative size of the polyembryonic plants is not controllable. It has to do with the size of the cotyledons with which each plant from a single seed is provided. This will be considered later.

Clearing and Staining. In order to study the venation of Citrus leaves, it was necessary to devise a method of clearing and staining so that the minutest bundle endings might be readily seen under the microscope.

An examination of the literature revealed only one or two methods for clearing leaves. DeVries (1878), working with potato leaves, gives the following directions for clearing: "Decolorize them in alcohol, treat for a longer time with caustic alkali, and finally wash with water and a dilute solution of acetic acid. The leaf is then put in glycerine and upon standing some time becomes transparent." The method described by Stevens (1907) involves, likewise, the use of alcohol for extracting the chlorophyll. This is wholly inadequate for the thick, leathery leaves of the Citrus species. Therefore a new method was perfected which, with a little modification, was found to be practical in clearing all kinds of leaves.

Entire leaves, if small, or portions of large leaves were placed in a basin of water and boiled for some time. This treatment is necessary in order to remove water-soluble materials, such as tannin, which prevent an even distribution of the stain.

After the leaves had taken on a water-soaked appearance, they were transferred to a side-tube suction flask. This contained 100 cc. of 85 percent nitric acid.[1] The flask was tightly stoppered, and, by means of a Richard's air pump, a partial vacuum was maintained. Such a process accelerated the penetration of the acid and at the same time drew off the escaping fumes which, if allowed to remain, caused a blistering of the tissue.

The leaves were allowed to remain in the acid in vacuum from twenty-four to thirty-six hours or until they had become transparent. They were then washed in running water for six hours, dehydrated, and introduced into the stain. After trying a number of stains, *e.g.*, fuchsin, orange G, safranin, iodine green, neutral red, and methylene blue, the last was found to be by far the most satisfactory. An alcoholic 1 percent solution was found to stain the lignified tissue a deep blue, leaving the remaining parts relatively free from stain. The usual time allowed for staining was twenty-four hours. Decolorizing in acid alcohol was often necessary.

Then, following complete dehydration in absolute alcohol and subsequent clearing in xylol, the leaves were mounted in castor oil. This medium has the following advantages over balsam or glycerine for this work: (1) it penetrates more readily than balsam and is easier to handle, and (2) it will not take up water as will glycerine. These mounts were made only for temporary usage, yet they are still in good condition at the end of two year's time. If permanent mounts are desired, balsam may be used to seal the edges.

Modification of the Method for Thin Leaves. The method described above was employed in treating thick, leathery leaves. For thin, succulent ones, like grape leaves, the following modifications were necessary to insure success:

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[1] Benedict (unpublished) used concentrated nitric acid for clearing. After allowing the leaves to remain in the acid for a while they were removed to a slide and floated in glycerine. The slide was then heated over a Bunsen flame until the leaves were transparent. This method is objectionable because the leaves usually go to pieces.

1. The nitric acid must be diluted to about 50 percent.
2. The length of time necessary for clearing is shorter.
3. The stain should be diluted to 0.5 percent.
4. Complete penetration of the stain is effected in about 12 hours.

In other respects the procedure is the same as that given for the heavier leaves.

Examination of Material and Determinations. All the determinations of vein-islets were made by projecting the magnified image of the stained vascular system by the use of a micro-projection apparatus. This consists of a horizontally placed microscope through which a stream of light, derived from an electric arc, is permitted to pass. The section to be studied was placed upon the stage of the microscope. The light is absorbed by the stained bundles and they appear as a shadow upon white paper. The paper was always placed at the same distance from the ocular of the microscope in making determinations. Adjustment was made so that a piece of paper 75 mm. square would just take a projected image from 4 sq. mm. of leaf surface. Now by marking with a lead pencil in the center of each of the vein-islets, the number of islets in 4 sq. mm. of leaf surface was quickly and accurately determined. The average area of each islet, then, was computed by dividing 4 sq. mm. by the number of vein-islets in that area.

PERCENTAGE OF SEEDS PRODUCING POLYEMBRYONIC SEEDLINGS

Webber (1900) states that the majority of Citrus seeds produce polyembryonic seedlings. Aside from this statement no data are available on this point. Therefore some germination tests were conducted as follows: tin basins were filled nearly full of sawdust which had been previously washed and sterilized. Upon this a layer of cheesecloth was laid and the seeds were placed upon the cheesecloth. Then another layer of cloth and more sawdust were added. The seeds prior to planting were treated for six hours in a 7 percent solution of calcium hypochlorite. These precautions eliminated a good many of the contaminating fungi.

It was found that seeds subject to desiccation in storage for only ten days had lost their vitality. Therefore all the seeds for this test were taken from the grapefruit and planted immediately. Germination began after a period of five days and continued for fourteen days.

Table 1 is a summary of these tests.

The results show that a high percentage of the fresh seeds are viable. Despite the precautions taken against contamination, the majority of the seeds which did not germinate were infected. This was indicated by the fact that these seeds rotted, and also by the fact that a higher percentage of germination was obtained when the seeds germinated in pure culture.

TABLE 1. *Percentage of germinating seeds of Citrus grandis producing polyembryonic seedlings*

Total No. Seeds Used in Test	Percentage of Seeds that Germinated Producing				Total Per-cent Ger-minated	Total Percent Producing Poly-embryonic Plants
	1 Hypocotyl	2 Hypocotyls	3 Hypocotyls	4 Hypocotyls		
888	48.82	37.8	5.9	4.2	92	43.18

The interesting facts to be noted, however, are that nearly half of the seeds which germinated produced more than one plant each, and that only four seeds out of the entire lot produced four seedlings each and the great majority produced but two each.

INFLUENCE OF CERTAIN FACTORS ON VENATION

Schuster (1908) has contributed more of real value as regards the effects of environmental conditions upon venation than any other writer upon this subject. Benedict (1915) has verified some of these findings in respect to *Vitis vulpina*. Zalenski (1902) did not confine his comparisons to a single species, so that hereditary variations were not eliminated. All the work, however, published by these writers shows that there are certain variations attributable to environmental causes. These should, then, so far as possible, be eliminated before a comparison of the venation of polyembryonic seedlings is undertaken.

UNIFORMITY IN SIZE OF VEIN-ISLETS IN DIFFERENT PARTS OF A LEAF

Schuster (1908) points out that there is a marked uniformity in the size of the vein-islets in all parts of the leaf except near the midrib and at the apices. The shape of the vein-islets in leaves of the grapefruit varies greatly in different regions of the leaf. Near the midrib they are long, narrow, and rectangular, and gradually become more nearly circular toward the periphery. Here a coalescence of the secondary bundles occurs, and the vein-islets are quite circular. Because of this difference in shape it was thought advisable to make determinations as to the size of the islets in the regions of the periphery and of the midrib respectively. The results of these findings are summarized in table 2. Sixty leaves were examined and four determinations were made from each region in each leaf. Hence, the mean averages represent a total of 240 determinations. In every table following, the figures representing the size of the vein-islets of a single leaf are the averages of four determinations.

The data show that the size of the vein-islets is independent of their shape or place in the leaf. Therefore, in later work, determinations were taken from either the periphery or the region of the mid-rib.

TABLE 2. *Showing uniformity of vein-islet area in different regions of the same leaf (Citrus grandis)*

Leaf No.	Number of Vein-islets in 4 Sq. Mm. of Leaf Surface		Size of Vein-islets (Sq. Mm.)	
	Midrib	Periphery	Midrib	Periphery
1	12	12.5	.3333	.3250
2	13	13	.3123	.3123
3	13	11	.3123	.3666
4	10	11	.4000	.3666
5	12	13	.3333	.3123
6	14	16	.2894	.2500
7	12	13	.3333	.3123
8	13	13	.3123	.3123
9	8	8	.5000	.5000
10	11	11	.3666	.3666
Average	11.8	12.1	.3492	.3423
Average 50 additional leaves	10.5	10.6	.3809	.3775
Mean average 60 leaves	10.7	10.9	.3756	.3714

INFLUENCE OF AN INSUFFICIENT FOOD SUPPLY UPON THE SIZE OF VEIN-ISLETS

To some of the pots in which the Citrus seedlings were grown, no fertilizer was applied. The soil was sandy and very poor in humus content. These plants were noticeably below the normal size of the other two-year-old seedlings. They were also chlorotic, and on the whole gave a very sickly, stunted appearance. During the course of the summer, some of these plants sent their roots through the drain hole in the pot and established themselves in the soil upon which the pots were placed. These plants at once responded to the effects of an adequate food supply and grew rapidly. The new leaves had a perfectly normal color and size, and the old leaves regained in part a normal color.

Mature leaves were taken from these stunted plants and the venation was studied. The results are given in table 3, where the size of the vein-

TABLE 3. *Influence of insufficient food supply upon size of vein-islets of leaves of Citrus grandis*

Plant Number (2 Leaves per Plant)	Number of Vein-islets in 4 Sq. Mm.		Size of Vein-islets (Sq. Mm.)	
	Chlorotic	Healthy	Chlorotic	Healthy
1	12	10	.3333	.4000
2	13	10.5	.3123	.3800
3	13	10.2	.3123	.3956
4	10	11.5	.4000	.3570
5	12	11.5	.3333	.3333
6	14	12	.2989	.3333
7	12	12	.3333	.3752
8.–	8	10.8	.5000	.3666
9	11	11	.3666	.3800
10	10	10.5	.4000	.3405
Average for 10 plants	11.5	11.00	.3590	.3661

islets of leaves from normal plants is compared with the same character in leaves from the chlorotic ones.

While the vein-islets in healthy leaves showed an average size slightly smaller than the size of those in the chlorotic leaves, the difference is well within the range of variations noted in individual leaves in each series. Hence the conclusion is warranted that vigor and nutrition have no measurable effect upon the size of the vein-islets (figs. 1 and 2).

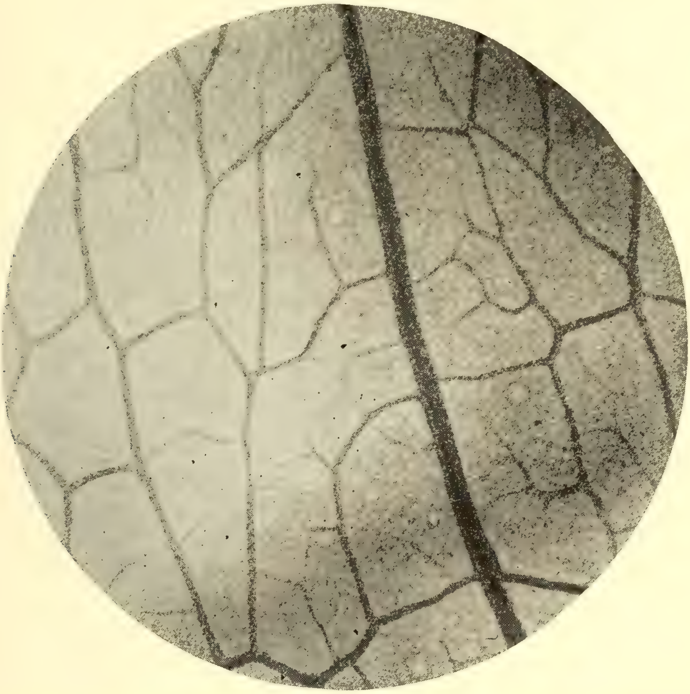

FIG. 1. Photomicrograph showing the venation in a mature chlorotic leaf of *Citrus grandis* measuring 16 mm. in short diameter. × 30.

INFLUENCE OF THE SIZE OF LEAVES UPON THE SIZE OF VEIN-ISLETS IN CITRUS GRANDIS

One of the most common variations with which one meets is in the size of mature leaves borne by a single plant. The polyembryonic Citrus seedlings are no exception. Experiments were accordingly made to ascertain whether this variation in size of the leaf was in any way correlated with the size of the vein-islets.

Only mature leaves were used, but the size of the leaves varied from 10 millimeters up to 68 millimeters in width. Only the short diameter of each leaf is given, taken at right angles to the midrib at the point of maximum width. The long diameter, *i.e.*, parallel with the mid-rib, was usually two times the short diameter. The results are given in table 4. The table shows that the size of the vein-islets in these leaves is quite constant irrespective of the great differences in the size of the leaves.

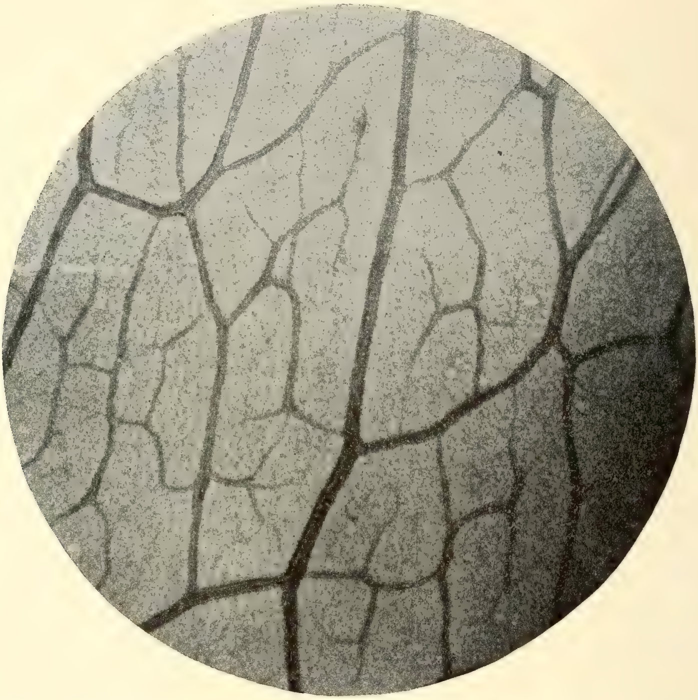

FIG. 2. Photomicrograph showing the venation in a mature healthy leaf of *C. grandis* measuring 68 mm. in diameter. × 30.

TABLE 4. *Influence of size of leaves upon size of vein-islets in leaves of Citrus grandis*

Leaf No.	Size of Leaf (Mm.), Short Diameter		No. of Vein-islets in 4 Sq. Mm. of Leaf Surface		Size of Vein-islets in Mm.	
	Small	Large	Small	Large	Small	Large
1	20	40	11.5	10.7	.3405	.3740
2	25	38	10	10	.4000	.4000
3	12	40	9.2	11	.4460	.3666
4	22	42	11.2	13.2	.3530	.3040
5	27	33	9.2	11.7	.4460	.3400
6	13	35	11.2	14	.3530	.2894
7	16	40	12.7	9.2	.3199	.4460
8	20	40	11.5	9.7	.3499	.4267
9	18	47	11.7	10.7	.3405	.3740
10	15	40	10.5	10.7	.3800	.3740
Average	18.8	39.5	10.87	11.09	.3728	.3694
Mean average for 25 leaves	17	45	10.7	11	.3740	.3666

Influence of the Maturity of the Leaves upon the Size of the Vein-islets in Citrus grandis

There seem to be no data published concerning the duration of time that the leaves persist upon grapefruit plants. Some of the plants at the greenhouse are now three years of age and no appreciable leaf drop has been noted. A leak of illuminating gas defoliated some of the plants of other species in this house, but the injury, if any, to the grapefruit was not sufficient to be noticeable. New growth is terminal and begins in the greenhouse early in March following a rest period.

In some of the preliminary work, which was done during the summer of 1916, it was noted that among five or more leaves taken from a single plant, one or sometimes two would occasionally show vein-islets very much smaller than those in the other leaves. In order to determine the meaning of this discrepancy the following method was employed. Beginning with the topmost leaf and proceeding downward to the last leaf at the base, a series of leaves was taken which represented the oldest and youngest leaves upon the plant and a gradual gradient from one to the other. In most cases sixteen leaves comprised such a series, and their individual venation was determined. Table 5 gives the data from one series. The others were all comparable with this one.

TABLE 5. *Variation in size of vein-islets in leaves of Citrus grandis owing to different degrees of maturity*

Leaf No.	Diameter of Leaves Mm.		No. Vein-islets per Unit Area (4 Sq. Mm.)	Size of Vein-islets (Sq. Mm.)
	Short	Long		
1	8	23	103	.0388
2	10	28	98	.0408
3	11	30	72	.0554
4	18	36	67	.0597
5	24	50	54	.0741
6	32	60	41	.0975
7	39	80	27	.1483
8	30	70	20	.2000
9	48	110	13	.3123
10	45	82	12	.3333
11	20	35	11.5	.3499
12	40	70	10.8	.3860
13	30	55	9	.4500
14	35	50	9.5	.4730
15	30	25	10	.4000
16	20	40	12	.3333

Here for the first time a positive correlation is evident (figs. 3 and 4). The most immature leaf in which a differentiation of the bundles has occurred[2] contains the greatest number of vein-islets per unit area. Furthermore, it appears that with increasing maturity there is a corresponding increase in the size of the vein-islets until maturity is reached.

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[2] Nägeli (1855) and Prantl (1883) have shown that the nervature of certain dicotyledonous leaves is not completely performed in the bud, but that new veins arise as the leaf expands. This is the case in leaves of *Citrus grandis.* The smallest leaves in which the bundles would take a differential stain were those measuring not less than 8 millimeters (short diameter).

Twenty immature leaves of varying size showed a very marked variability in the size of the islets, while in the mature leaves they tended to be constant. The immature leaves are distinguishable from the mature also by their lighter green color, and by a more resinous feel.

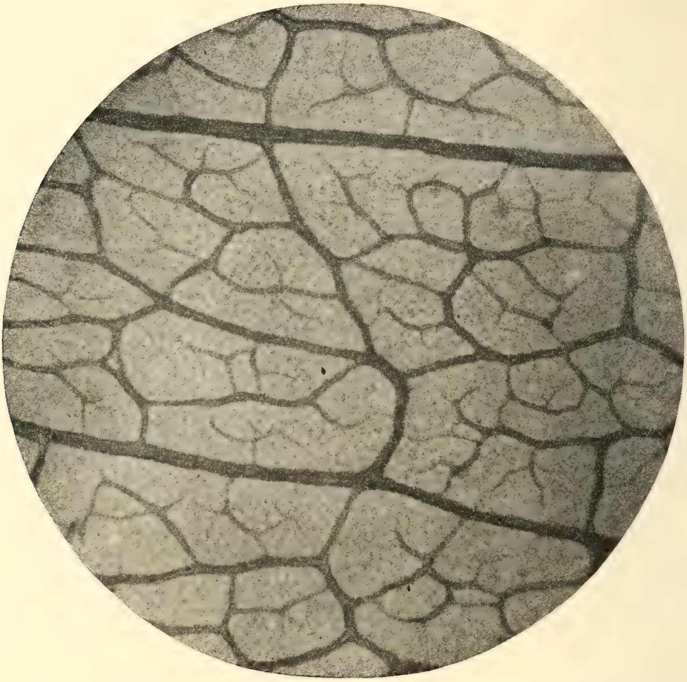

FIG. 3. Photomicrograph showing the venation in an immature leaf of *Citrus grandis*. × 30.

One may conclude, therefore, that the main skeleton work of the vascular system in the leaf is laid down quite early; that little additional differentiation takes place after this as the leaf continues to expand, and that the spaces between the bundles consequently increase in size until the leaf is mature. The bundles themselves become much larger in cross section as the leaf advances toward maturity. In very young leaves the stained bundles appear as fine blue threads in striking contrast to the large, rigid veins found in mature leaves. One thing which seems inexplicable is the fact that the plant seems to predetermine how much differentiation shall be allowed this or that leaf, so that at maturity the number of intersecting veins in a unit area of leaf surface shall be more or less nearly constant, independent of the size of the leaf.

VARIATION IN THE SIZE OF VEIN-ISLETS IN POLYEMBRYONIC SEEDLINGS OF CITRUS GRANDIS

Since some of the more variable factors have been eliminated, the way seems clear to take up the principal question: Is there any difference between the polyembryonic seedlings as regards the character of venation?

Only mature leaves were used. The larger plant was labeled *A* and the smaller one *B*, when two were produced from a single seed. When the plants were equally large and vigorous, the lettering was purely arbitrary.

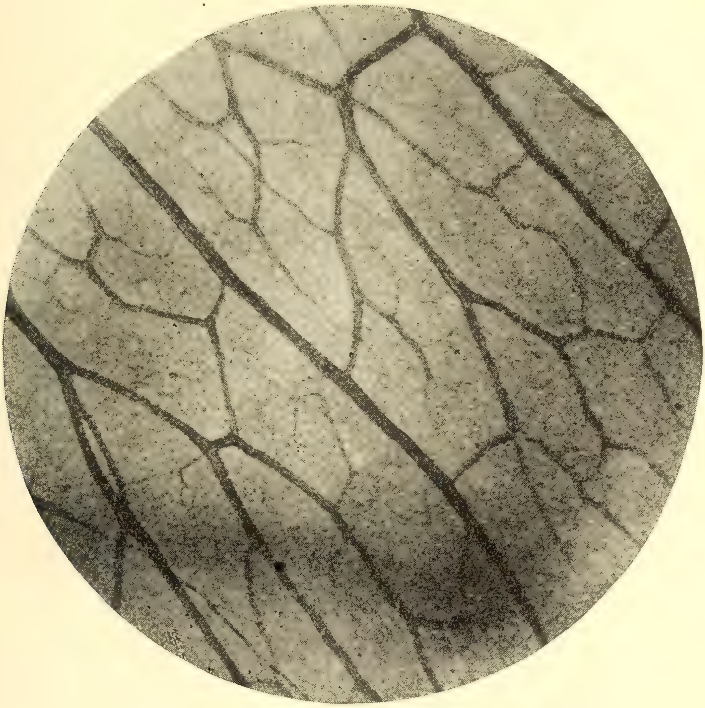

FIG. 4. Photomicrograph showing the venation in a mature leaf of *Citrus grandis*. × 30.

Altogether 100 leaves were examined from each of the *A* and *B* series. This represents 20 plants and 5 leaves from each plant.

The only conclusion which these data warrant is that, so far as the venation test for senility is concerned, it has failed to reveal any essential differences between polyembronic seedlings of *Citrus grandis*. While there is no way of knowing positively the number of polyembryonic plant pairs used in this study composed of individuals having apogamous and gametic origin respectively, yet there can be little doubt but that the leaves from such pairs were encountered and studied in one phase or another of the work. Since no marked differences in vein-islet area were discovered, the following possible explanations are offered: (1) Since the age of the parent plant yielding the seed is not known, it is possible that the difference between parent and seedling is not great enough to be measurable by this test. (2) The venation character may not be an indication of age differences in this species.

TABLE 6. *Size of vein-islets in leaves from polyembryonic seedlings of Citrus grandis*

Leaf No.	Number of Vein-islets in 4 Sq. Mm.		Size of Vein-islets (Sq. Mm.)	
	Plant *A*	Plant *B*	Plant *A*	Plant *B*
1	10	9.2	.4000	.4431
2	10.5	10.2	.3800	.3920
3	10.8	11	.3750	.3666
4	10	11	.4000	.3666
5	11.5	8.8	.3499	.4605
Ave. 1st pair plants	10.56	10.04	.3809	.4057
1	13.7	10.5	.3040	.3820
2	14	14	.2894	.2894
3	13.5	12	.2955	.3333
4	11.7	13.7	.3500	.3050
5	11.5	11	.3499	.3666
Ave. 2d pair plants	12.88	12.24	.3177	.3352
1	13	13	.3123	.3123
2	13.7	15.8	.2999	.2558
3	10	14	.4000	.2894
4	11.5	13	.3499	.4000
5	——	10	——	.4000
Ave. 3d pair plants	12.05	13.16	.3405	.3315
1	10	10.2	.4000	.3820
2	8.8	10.7	.4605	.3750
3	10.2	9.8	.3920	.4160
4	11	10	.3666	.4000
5	10	11	.4000	.3666
Ave. 4th pair plants	10.2	10.34	.4038	.3879
1	10.2	10	.3920	.4000
2	9.8	10	.4270	.4000
3	11	11.8	.3666	.3400
4	11	11	.3666	.3666
5	10.8	10.2	.3741	.3920
Ave. 5th pair plants	10.56	10.60	.3852	.3797
Mean ave. for 25 leaves—5 pairs of plants	11.29	11.15	.3655	.3680
Mean ave. for 100 leaves—25 pairs of plants	11.1	11.0	.3624	.3666

The reasons that may be advanced in favor of these suggestions are:

1. The grapefruit was not grown in Florida on an extensive commercial scale prior to the big freeze of 1894.[3] At most, then, the bearing trees are probably not more than twenty-five years old. Hence the gametic seedlings, which are assumed to be rejuvenated, may not be far enough removed in age from the apogamous seedlings, which are senile to the same degree as the parent, to show a measurable difference in the size of vein-islets.

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[3] Bailey, L. H., Encyc. Hort. **2**: 782.

Opposed to this is the fact that Benedict (1915) reports an evident difference in cases in which the difference in age was only approximately three years. A great deal of his data are derived from plants showing an age difference of no more than ten to twenty years.

2. It is possible that in *Citrus grandis* the venation character is not correlated with age. Yet Benedict found such a correlation in twelve species of woody plants, and he states that he believes it to be of quite universal application.

A third possible explanation is given in the discussion of results.

NUCLEO-CYTOPLASMIC RATIO

Inasmuch as the polyembryonic seedlings showed no differences from a morphological standpoint, it seemed advisable to try one other test for senescence—the nucleo-cytoplasmic ratio.

To Hertwig (1903) and Minot (1908) we are indebted for the hypothesis that there is a definite correlation between the relative volume of nucleus and the volume of cytoplasm in cells as an organism passes from youth to old age. Minot holds that the volume of the nucleus is much larger in proportion to the volume of cytoplasm in cells of a young organism, and that, as age advances, the volume of cytoplasm gradually increases with a corresponding decrease in the volume of the nucleus.

Hertwig held a view diametrically opposed to that of Minot.

Conklin (1912) contends that such a ratio does not hold even in cells of the same tissue, for the ratio varies with the different phases of mitosis.

The meristematic tissue of the root tips of polyembryonic seedlings was used. The seeds were germinated in pure culture, in test tubes on agar. They were first sterilized by removing the outer husk and allowing the naked seeds to stand in 7 percent hypochlorite of lime for five hours.

The hypocotyls were allowed to grow until they were from ten to fifteen millimeters long. At this time the polyembryonic seedlings from single seeds showed differences in length of the hypocotyls in many cases. Those seedlings which showed this difference were the only ones used in this study, and as in the previous test the larger one was termed *A* and the other *B*.

Treatment. The root tips were killed in 1 percent chrom-acetic acid solution. They were allowed to remain in this solution for twenty-four hours, at the end of which time they were washed in running water for an equal period. The subsequent treatment for fixing and clearing is that recommended by Chamberlain (1915) for root tips. The sections were cut longitudinally 10 μ in thickness and stained with Heidenhain's iron-alum haematoxylin.

All determinations of the nuclear and cell diameters were made under the oil immersion objective. Inasmuch as a very rapid vacuolization of the cytoplasm occurs as the cells become more and more differentiated, no better method of insuring comparable volumes could be hit upon than by taking those cells in the cortex at the same distance from the base of the root cap and which contained nuclei that were round in section. The volume of the cytoplasm was assumed to be that of the cell minus the volume of the nucleus. The volumes were computed on the basis that the cells were cylinders, and the nuclei spheres.

Fourteen root tips from each of the *A* and *B* hypocotyls were examined. In table 7, these data are summarized. The figures representing the volume of nucleus and cytoplasm, respectively, for each root tip, are averages of fifteen to twenty-five determinations.

TABLE 7. *Summary of determinations of nucleo-cytoplasmic ratios made from each pair of polyembryonic root tips of Citrus grandis*

No. of Root Tip.	Volume Nuclei (Cu. Microns)		Volume Cytoplasm (Cu. Microns)		Nucleo-cytoplasmic Ratio	
	A	B	A	B	A	B
1	166.89	187.05	626.06	544.13	1 : 3.9	1 : 2.9
2	195.02	187.05	881.04	544.13	1 : 4.4	1 : 2.9
3	166.89	164.52	1,168.47	826.81	1 : 7	1 : 5
4	187.05	187.05	1,397.27	1,265.36	1 : 7	1 : 6.7
5	166.89	142.03	1,168.47	868.23	1 : 7	1 : 6.1
6	130.58	187.05	1,189.14	1,113.80	1 : 9	1 : 5.9
7	187.05	164.52	778.67	566.66	1 : 4	1 : 3.4
8	142.03	194.99	687.64	994.93	1 : 4.8	1 : 5
9	160.69	180.75	1,123.38	658.11	1 : 7	1 : 3.9
10	226.18	191.94	1,421.63	672.22	1 : 6	1 : 3.9
11	180.75	154.20	1,428.39	843.04	1 : 7.9	1 : 6
12	194.99	140.26	686.72	1,430.70	1 : 3.5	1 : 10
13	166.97	157.08	1,053.67	1,414.04	1 : 6.3	1 : 8
14	174.59	173.19	1,396.88	1,267.60	1 : 8	1 : 7
Mean . . .	174.75	171.52	1,069.60	859.30	1 : 6.3	1 : 5.0

The results show that the nucleo-cytoplasmic ratio as determined by this method is subject to considerable variation. This may be explained in part by the fact that the percentage of vacuolization of the cytoplasm at an equal distance from the apical cell of the meristem is not the same in every root tip. It is, obviously, impossible to make the necessary corrections, for there is no way of knowing the degree of differentiation that has taken place. The only possible way to overcome this difficulty is to take mean averages of many determinations. The mean averages for fourteen root-tips examined show that in a measure this error has been overcome. It also shows that the nucleo-cytoplasmic ratio is not appreciably different in the *A* and *B* root-tips. Certainly the difference is not as great as the individual variations in either case.

SOME PHYSIOLOGICAL CONSIDERATIONS

Since the two tests which have gone before have revealed no apparent difference between the seedlings arising from a single seed, the question then naturally arises: Do the polyembryonic seedlings from single seeds show any differences in size and vigor? If there is a difference, to what is it due?

Webber (1900) found that, when *Citrus aurantium* was crossed with *C. trifoliata*, among polyembryonic seedlings one plant would usually show intermediate characters common to both parents. This seems to leave no doubt but that such a plant had its origin from the fertilized egg. This hybrid, furthermore, was usually larger than the other plants according to Webber, who attributes the difference in vigor to the beneficial results of crossing. This is explicable on the ground that when hybridization is effected between parents of distinctive characters, new combinations of characters often result which are more favorably adapted to the environment than are those of either parent. This greater vigor has often been mistaken for an indication of rejuvenescence.

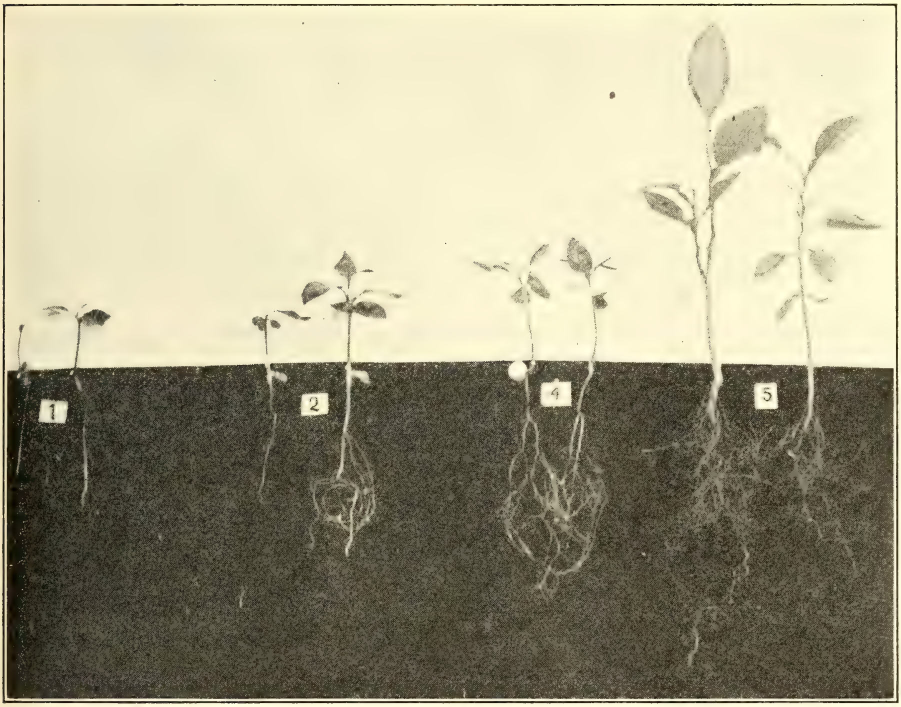

FIG. 5. Photograph showing 4 pairs of polyembryonic seedlings of *C. grandis*. Nos. 1 and 2 show polyembryonic plants of unequal size. The relative size of attendant cotyledons in no. 2 are easily seen. The size of cotyledons is evidently correlated with the size of the plants. No. 4. Polyembryonic plants of equal size and vigor (one-year-old seedlings). No. 5. Polyembryonic plants of equal size. They are three years old and chlorotic.

Among all the seedlings of *Citrus grandis* grown in the greenhouse, there were no true hybrids, as far as the writer observed. Yet in many cases two plants from a single seed showed differences in size. In other cases both plants developed equally well. Of 36 pots containing two seedlings each, 40 percent contained seedlings of substantially equal size and apparent vigor. The remaining 60 percent showed plants of unequal size in all gradations.

At the time the germination tests were made, it was a matter of common observation that each polyembryonic seedling had its pair of cotyledons attached. In case three or four embryos developed from a single seed, some of the last to germinate had cotyledons not much larger than a pin head. On the other hand, some of the others were subtended by cotyledons that comprised the major portion of the entire seed. Although no specific data were taken on this point, it seems only reasonable to conclude that the size of the germinating seedling is directly correlated with the size of the cotyledons, or the amount of food. The general observations substantiate this view (figs. 5 and 6).

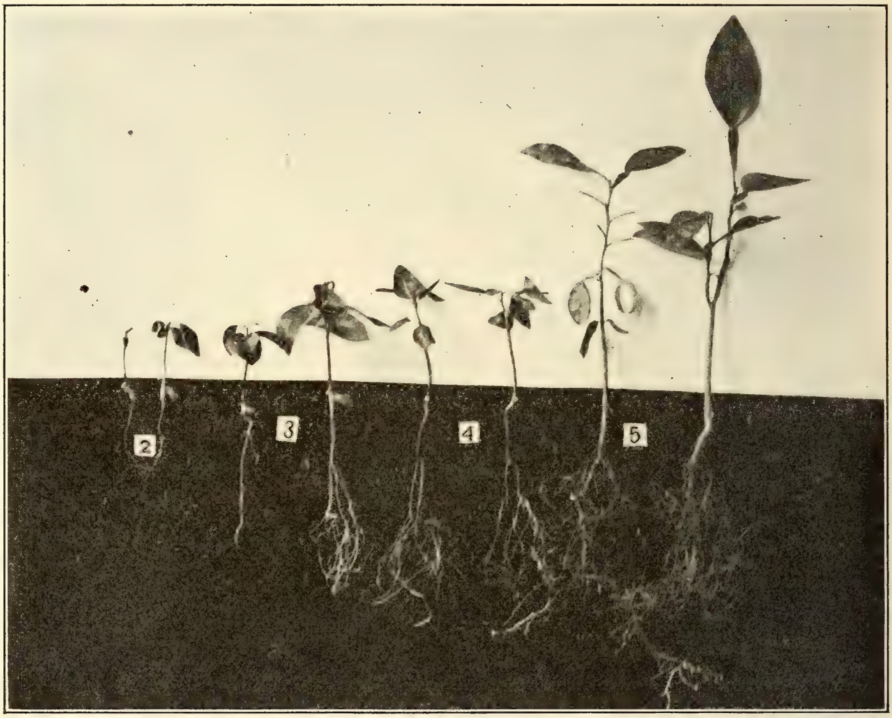

FIG. 6. 4 pairs of polyembryonic seedlings of varying age and size. No. 2. Polyembryonic seedlings 5 months old, of unequal size and vigor. No. 4. Polyembryonic seedlings 11 months old, of equal size and vigor. No. 5. Polyembryonic seedlings 3 years old, of about equal size and vigor.

Strasburger (1878) showed that sometimes the apogamous embryo develops first and much more rapidly than the one arising from the fertilized egg; that in some cases the former, wholly or partially, suppresses the development of the latter. Osawa (1912) states that it is very common for as many as nine embryos to be present in the embryo sac, yet only rarely do more than four develop.

These observations all tend to show that the relative size and vigor of polyembryonic plants is primarily a matter of priority of development and food content of the cotyledons. The embryo, whether gametic or apogamous, which has the initial start and the greater reserve of food will probably produce the larger plant. In those cases in which the embryos develop simultaneously, we should expect to have plants of equal size and vigor.

Discussion of Results

The important question, of course, is this: Do the results have any bearing upon the question of rejuvenescence in plants?

Rejuvenescence in the sense in which Minot (1908), Child (1915), and Conklin (1913) define the term will be used here. They hold that rejuvenescence is a condition of an organism indicated by metabolic activity increased over what it was prior to the change; and that the methods by which such an increased rate may be indicated vary, but the process is always the same—a partial or entire "undifferentiation," or unloading of differentiated products which interfere with maximum metabolism. In a general way it may be said that differentiation and growth are gradual processes, usually having their origin in a single cell. This passage from the simple to the complex in the life cycle of an organism is invariably accompanied by a gradual retardation of the rate of metabolic activity, which finally ceases, and death ensues. Minot aptly says that death is the price paid for differentiation. On the other hand, the process of "undifferentiation" must and undoubtedly does take place. Just what the process is, and how it may be effected, is still largely a matter of conjecture.

It has been quite generally assumed that sexuality is essential to rejuvenation. Child has shown that complex organisms may be partially rejuvenated by a period of partial starvation, and Conklin calls attention to the rejuvenation which apparently takes place in frogs and other animals after a period of hibernation, comparable to the starvation period of Child. He also suggests that such periods of rest and encystment shown by many animals and plants are directly correlated with partial rejuvenation processes. Furthermore, Child has shown that apogamous reproduction is just as effective as gametic reproduction in securing rejuvenescence. Indeed, this would seem to be the case in plants like the banana whose sexual apparatus for unknown periods of time has been inoperative, and yet in which there has been no indication of the plant's "running out."

Now all the evidence gathered in the study of polyembryonic Citrus seedlings seems to indicate that, if we admit that rejuvenescence has occurred at all, it has occurred to the same degree in apogamous and gametic plants. And in the light of the more recent work of Child (1915), Conklin (1913), and Woodruff (1914), we may conclude that sexuality is not necessary to rejuvenation.

That the process of "undifferentiation" and sexuality often occur at approximately the same period in the life cycle of the organism is not denied. Indeed, the reduction or "undifferentiation" of the organism to a single cell is essential before fertilization can occur, but by this reduction rejuvenescence is already effected independent of any fusion which may or may not occur later. Providing the necessary conditions for development surround the single-celled embryo, whether it is stimulated to activity by a sperm, or a needle prick, or certain physiological conditions, is of little moment so far as rejuvenescence is concerned. It is not impossible that the similar physiological conditions surrounding the polyembryonic embryos in their development may explain the similarities noted in the seedlings. The primary function of sexuality it would seem is the production of new character combinations in the offspring through the redistribution of distinctive ones found in the parents. This view is substantiated by the work of Jennings (1912) and Woodruff (1914), who show that protozoa are potentially immortal, and that sexuality serves the sole purpose of producing favorable variations.

Summary

1. Germination tests showed that 43.18 percent of the seeds of *Citrus grandis* produce polyembryonic shoots.
2. A new method for clearing and staining leaves is described.
3. The size of vein-islets is not correlated with the shape and location of the islets in leaves of *C. grandis*.
4. The size of the vein-islets is directly correlated with the maturity of the leaf. From the most immature to the fully matured leaves there is a gradual increase in the size of vein-islets.
5. In mature leaves the size of the vein-islets is quite constant, independent of the size of the leaf.
6. The size of vein-islets in mature leaves from chlorotic, stunted plants is the same as in mature leaves from healthy plants.
7. The size of vein-islets revealed no difference between polyembryonic seedlings arising from a single seed. No doubt the individuals composing some of these polyembryonic pairs were of gametic and apogamous origin respectively.
8. The nucleo-cytoplasmic ratio, as determined in the meristem of the root tips from polyembryonic plants, is essentially the same in gametic and apogamous plants.
9. Difference in size of polyembryonic seedlings seems to be a matter

primarily concerned with priority of development of the embryos, and not directly correlated with sexuality.

10. Rejuvenation seems to be effected equally well through apogamous and through gametic reproduction.

The writer wishes to express his indebtedness to Professor Lewis Knudson for valuable suggestions, and for placing at his disposal the Citrus seedlings with which this study was made. He also acknowledges the helpful suggestions of Dr. L. W. Sharp.

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A STUDY OF SOME FACTORS IN THE CHEMICAL STIMULATION OF THE GROWTH OF ASPERGILLUS NIGER

Robert Aaron Steinberg

The distinction between "nutrients" and "stimulants" has been the subject of intensive study since synthetic culture media of known composition have come into use. It was early observed that substances which were considered to be by no means essential constituents of protoplasm might still produce a marked increase of growth. The phenomena of so-called "chemical stimulation" due to the addition to a nutrient solution of substances not regarded as essential for growth are apparently of general occurrence. Such phenomena have been described for bacteria (16), fungi (57), algae (49), and the higher plants (25, 7, 33). These studies for the most part had for their aim to ascertain the effect of the substance in question on the growth and on respiration. I have reported elsewhere (64) on evidence that substances may be dissolved in sufficient quantity from the glass of the cultural flasks to cause a partial or even a maximum acceleration of growth. I have also presented evidence that the amount of growth as well as the concentration of "stimulant" necessary for its production varies with the strain of *Aspergillus niger* used (65). I shall report here on further experiments testing the significance of the degree of acidity of the culture medium and the effect of varying its iron or zinc content singly or together. These results are obtained by the use of a method of purifying the culture medium not hitherto used in such physiological experiments. Lastly I have attempted to correlate the facts in the literature of "chemical stimulation" with some of the newer data on the problems of nutrition and growth obtained by other methods.

The studies of Pasteur (51) on the alcoholic fermentation of yeast led to extensive investigations of the food requirements of the fungi. It was determined by Pasteur in the course of his investigations that yeast could grow in an aqueous sugar solution containing ammonium tartrate and yeast ash. Beyond ascertaining that the yeast ashes could not be replaced by magnesium phosphate and a few other salts, the matter was dropped. No attempt was made systematically to investigate the elements known by him to be present, from Mitscherlich's (38) analyses, in the yeast ash. The analyses referred to are shown on the following page.

The continuation by Raulin (54), a student of Pasteur, of these studies on the components of the yeast ash in relation to the growth of another fungus, "*Ascophora nigrans*" (*Aspergillus niger*), soon followed. Raulin

Composition of Ash

	Top Yeast	Bottom Yeast
H_3PO_4	41.8%	39.5%
K	39.8	28.3
Na	—	—
$CaHPO_4$	2.3	9.7
$MgHPO_4$	16.8	22.6
SiO_2	tr.	—

substituted various compounds, singly and combined, for the yeast ash in the Pasteur nutrient solution and determined the extent of growth as represented by the dry weight of the tissue formed. The "most useful" substances in the order of their importance were, according to Raulin: ammonium phosphate, potassium carbonate, magnesium carbonate, ammonium sulphate, and manganese carbonate.

In 1869 a continuation of these studies was published by Raulin (55), and almost simultaneously appeared an important contribution by A. Mayer (38).

Raulin had continued his studies on *Aspergillus niger*, arriving at a nutrient solution "essai type" which contained, as he held, all the elements "essential" for the growth of this organism in the proper proportions. The omission of any component of his "essai type" solution resulted, he stated, in a large decrease in growth. I repeat the formula as a matter of historical interest: water 1,500 g., cane sugar 70.00 g., tartaric acid 4.00 g., ammonium nitrate 4.00 g., ammonium phosphate 0.60 g., potassium carbonate 0.60 g., magnesium carbonate 0.40 g., ammonium sulphate 0.25 g., zinc sulphate 0.07 g., iron sulphate 0.07 g., potassium silicate 0.07 g.

As a result of his studies on the "normal" development and respiration of a yeast, Mayer (38) had arrived, however, at a much simpler nntrient solution, containing, if we exclude from consideration the water and cane sugar, but four compounds as compared to the nine entering into the composition of the nutrient solution as worked out by Raulin. The elements Mayer considered "essential" are present in this solution in the proportions most favorable for "normal" development and respiration. Moreover, these proportions, Mayer points out, closely approximate those found in the yeast ash. The formula of the Mayer solution is: water 1000.0 g., cane sugar 150.0 g., ammonium nitrate 10.0 g., monopotassium phosphate 5.0 g., magnesium sulphate 2.5 g., calcium phosphate ($CaHPO_4$) 0.5 g.

A comparison of these two formulae with respect to the elements present shows a substantial agreement. Both solutions contain hydrogen, oxygen, carbon, nitrogen, phosphorus, sulphur, potassium, and magnesium. Raulin included in addition zinc, iron and silicon; Mayer, calcium only.

With respect to the necessity of silicon the evidence is as yet indecisive. Calcium has, however, been shown by Benecke (3) not to be an "essential" element in the nutrition of fungi.

The necessity of iron for the fungus has been a much disputed question. That iron is an "essential" participant in the metabolism of the fungus is the opinion of Molisch (45). Benecke's (3) experimental results, as he himself points out, are inconclusive. More recently Currie (11) has concluded that the exclusion of iron from the nutrient solution is without effect on the growth and reproduction of *A. niger*.

Von Nägeli (46) concluded that certain substitutions are possible. Thus, he thought, rubidium or caesium could be used in place of potassium; and calcium, barium, or strontium in place of magnesium. That Nägeli was in error has been shown by Benecke (3), inasmuch as in the entire absence of either potassium or magnesium he could obtain no growth. Benecke found, however, that partial replacement was possible: greater growth took place but the cultures did not fruit. This non-sporulation is, at least in the case of rubidium and caesium, not to be confused with an analogous phenomenon accompanying "stimulation" (see below) however, but is simply due according to Sauton (61) to insufficient potassium and not to a "sterilizing action" of the caesium and rubidium.

The nutrient solution utilized by Pfeffer (52), which is quite similar to that worked out by Mayer, is being increasingly made use of in nutritional studies. It is perhaps the simplest of the nutrient solutions in use.

PFEFFER SOLUTION

Water	1000.0 g.
Cane sugar	50.0 g.
Ammonium nitrate	10.0 g.
Monopotassium phosphate	5.0 g.
Magnesium sulphate	2.5 g.
Ferrous sulphate	trace

As respects the action of the components of the nutrient solution, it was early partially recognized by v. Nägeli (46, 47) that the action of a compound on the growth of an organism depends on and increases with its concentration, and that the presence of other compounds can cause a modification of this action. Each compound, according to him, exercises a "specific action" which is apart from and without reference to its nutritive value. Every compound has an "optimum" concentration for growth; and a "schädliche" (toxic) concentration. The latter is higher in value than the "optimum." Both the "optimum" and the "toxic" concentration vary with the compound and with the particular organism used. In other words, the "optimum" concentration of any compound in a nutrient solution varies with the composition and concentration of each of the other compounds present and with the organism.

Much evidence has accumulated showing the specific effect of "poisons," "stimulants," etc., on the growth of organisms. That in sufficiently high dilution many "poisons" are not harmful, but may cause an acceleration of the life activities of the organism was apparently known to Braconnot (6) in 1845. Schulz's (62) studies on yeast demonstrated that the presence of such substances may result in increased respiration. The rôle of zinc in the nutrition of *A. niger* and other fungi was apparently first considered as an analogous phenomenon by Pfeffer (52). Richards (57) on the basis of a broad comparative study concluded that the action of zinc is shared by many other substances, such as iron, lithium, cobalt, nickel, fluorides, etc., and even alkaloids; and that zinc therefore cannot be regarded as more "essential" than many other elements.

Since the initial research of Raulin (55), by which was indicated the increased yield resultant upon the addition of zinc to cultures of *Aspergillus niger*, various other (approximately seventeen) ions have been shown to act in a similar manner. "The increased growth," to quote Pfeffer (53, p. 146), "appears to be due to a general power of reacting against injurious influences possessed by living organisms, for similar results are produced by ether, alkaloids, etc., not only upon growth, but also upon respiration and fermentative activity." Further: "A very strong poison produces its optimal stimulating effect when extremely dilute and growth may be retarded by doses above the optimum, while substances which act as poisons only when highly concentrated produce no perceptible result at all."

The acceleration of growth of *Aspergillus niger* may appear in the presence of very diverse carbon (57, 56) and nitrogen (57, 22) sources. The increase in respiration has been studied by Schulz (62) with yeasts; and Richards (58), Ono (49), and others have pointed out that the "economic coefficient" $\left(\frac{\text{dry-weight formed}}{\text{100 g. sugar used}}\right)$ is increased in the presence of zinc. The total acidity of the culture solution, it has been found by Currie (11), increases more rapidly in the "stimulated" cultures than in the controls. This condition is reversed after some time.

The increased growth involves (Fred, 16) an increased number of cell divisions.

We may next note the evidence from the literature for increased growth as dependent on the acidity of the culture medium. Attention has already been drawn by Mathews (37) to the fact that the hydrolytic dissociation of the salts of the heavy metals is an important factor in their action, as shown by their precipitating power. Similarly, Mines (44) has pointed out that as a result of hydrolytic dissociation the action of an electrolyte may be either increased or decreased. Of interest in this connection are certain experiments by Pantanelli (50), who, in his studies on the selective absorption of ions from single salt solutions by *Cystosira amentacca*, found that in practically every case the H-ion concentration of the solution increased considerably after an immersion of two hours. The action of the salts of the heavy metals, which upon hydrolytic dissociation give an increased acidity of the solution (see for example Denham, 13), will probably be found to have been over- or underestimated on this account.

Evidence for the action of the hydrogen ion on growth and reproduction has been many times presented, and a direct correlation of this effect with that of the ions exercising a "stimulative effect" is perhaps possible.

Raulin (55, p. 162) pointed out quite clearly that a definite optimum acidity or alkalinity exists for the growth of each organism, and further that the optimum reaction varies with the organism. The "Mucédinees" and the "Mycodermes," he stated, prefer an acid medium; the "végétaux-ferments" a neutral; and the "Infusoires" an alkaline medium. Raulin himself utilized tartaric acid in his nutrient solution in order to favor the growth of *Aspergillus niger* and to exclude "Infusoires." With high concentrations he reports a retardation or suppression of growth and spore formation.

It has come to be a commonplace that fungi as a rule prefer an acid, and bacteria an alkaline, medium. This is the basis of the conception of many of the early students of plant diseases that only fungi and not bacteria could cause plant diseases; the bacterial diseases being confined to animal organisms whose protoplasm is alkaline (see Smith, 63). Similar statements with regard to the acidity requirements of fungi and bacteria have been repeated with great frequency, as for example by v. Nägeli (46, p. 63), Pfeffer (53, p. 384), and Jost (23, p. 205), without as a rule any definite statements as to the actual effects of acidity on the growth of any one organism.

As respects the algae, we may note that Migula (43) observed that very slight increases in acidity cause an acceleration of the growth in length. Cell division, however, seems not to have been accelerated.

For the higher plants as grown in water culture, A. Mayer (39, p. 273), Pfeffer (53, p. 415), Jost (23, p. 92), and even Russell (60, p. 49) in 1917, refer to the effects of acidity only vaguely.

Within comparatively recent times *quantitative* experimental evidence on the relation of acidity to growth has begun to accumulate. The discovery of Kahlenberg and True (24) that the toxicity of acids depends on their ionization (*i.e.*, the resulting H-ion concentration) has been of great importance and significance. We need only consult Michaelis' (41) summary of the literature to realize the fundamental importance of the H-ion concentration for enzymatic action, etc.

Clark (9) states that for a number of acids the growth of *A. niger* varies with the acidity: ". . . ¼ to ⅛ the lethal concentration has a strong stimulating influence on the mycelial development, and tends to suppress or at least retard fructification" (p. 294). Unfortunately no yields are given, and the acidities are calculated from the ionization of the acids in water though added in this case to a sugar-beet decoction. Fred and Loomis (17) have studied the influence of the H-ion concentration of the medium on the reproduction of alfalfa bacteria. The optimum acidity they note corresponds to $p_H = 7.2$; at $p_H = 3.10$ no growth occurs. [$p_H = -\log_{10}$

(H^+), *i.e.*, the negative logarithm of the hydrogen-ion concentration in gram-ions.] Furthermore, they note the same phenomenon observed by Hoagland (20) with barley; the acidity of the medium is modified as a result of growth, increasing where the initial acidities were too low, decreasing where the initial acidities were too high. Hoagland found $p_H = 5.2$ the optimum acidity for barley plants.

There is also some evidence bearing out the view of Pfeffer (53, p. 487) that a specific limiting acidity is reached as the result of the growth, etc., of certain organisms. Michaelis and Marcora (42) have found it to hold true in the case of *Bacterium coli;* Clark and Lubs (10) with *Aspergillus niger;* and Ayers (2) with many streptococci. This fact is at the basis of the procedure used in the laboratory of the addition of $CaCO_3$ in lactic acid fermentation. As is similarly generally recognized (53) an analogous phenomenon occurs in acetic acid, and in alcoholic, fermentation; and probably also in butyric acid fermentation. It is, we may say, frequently the case that the growth of an organism is limited by the attainment of a definite concentration of some acid produced by its own metabolism.

Experiments on the relation of the acidity of the Pfeffer nutrient solution to the growth and fructification of *Aspergillus niger* under conditions known to exclude to a high degree the "stimulative" ions, were undertaken in order to obtain cultures directly comparable with zinc cultures.

Methods

In the following experiments the Pfeffer nutrient solution was used exclusively. The compounds employed in the preparation of this solution were Merck's "Reagent" ammonium nitrate and magnesium sulphate; and Baker's "Analysed" potassium phosphate and ferric sulphate; the water was redistilled through glass (the first and last quarters of the distillate are rejected). The cane sugar used is that sold under the proprietary name of "Crystal Domino" sugar. The use of other compounds is always noted in the description accompanying the experiments. Tests with *A. niger* showed that the results obtained with "Crystal Domino" sugar in nowise differed from those obtained with Merck's "Highest Purity" cane sugar. In both cases growth proceeded as well with as without the addition of a trace of iron salt; and in the presence of zinc a marked acceleration of growth occurred. The information was obtained on inquiry from the manufacturer that no coloring matter is added to this sugar in the refining process, and that this practice had gone out of use over thirteen years ago.

The flasks used were 150 cc. pyrex Erlenmeyers. They were cleaned by rinsing with concentrated sulphuric acid (Baker's "Special"), tap-water, lastly with distilled water, and inverted to drain dry. It may be noted here that when zinc is present in the glass of the cultural flasks (Jena glass) sufficient amounts may go into solution to cause partial or maximum "stimulation," as I have shown elsewhere (64).

RESULTS

1. The influence of a zinc glass (Jena) on the yield [Reproduced from (64)].
$MgSO_4.7H_2O$ (Kahlb.) Room temperature (18–23° C.). Period of growth ten days.

Jena		Kavalier Bohemian		Pyrex	
No Zinc	10 Mg. Zn/L	No Zinc	10 Mg. Zn/L	No Zinc	10 Mg. Zn/L
0.989 g.	0.980 g.	0.270 g.	0.924 g.	0.319 g.	0.940 g.
0.958	0.940	0.299	0.943	0.248	0.980
0.919	0.988	0.285	0.886	0.306	0.917
0.933	1.005	0.300	0.947	0.252	0.952
0.953	1.022	0.361	1.017	0.309	0.997
0.950	0.987	0.301	0.943	0.287	0.957

2. The influence of a zinc glass (Jena) on the comparative yield of two strains of *A. niger* (W and Y).
$MgSO_4.7H_2O$ (Kahlb.)

Strain W, Transfer 1			Strain Y, Transfer 1		
Pyrex		Jena	Pyrex		Jena
No Zinc	0.01 Mg. Zn/L	No Zinc	No Zinc	0.01 Mg. Zn/L	No Zinc
0.302 g.	0.602 g.	0.805 g.	0.516 g.	0.806 g.	0.763 g.
0.265	0.578	0.741	0.535	0.830	0.747
0.339	0.562	0.810	0.546	0.843	0.785
0.330	0.528	0.786	0.409	0.694	0.750
0.335	0.554	0.811	0.393	0.732	0.787
0.317	0.565	0.791	0.480	0.781	0.766

In Jena glass, it will be noted (experiments 1, 2) that the maximum growth occurs whether zinc is added or not.

The following experiment reproduced from a former publication (64) will also show that the method used in cleaning the flasks is entirely satisfactory. The flasks used for the cultures in the second column were previous to this experiment used three consecutive times for cultures containing 10 Zn/L and were cleaned as usual.

3. On the efficiency of the method for cleaning the cultural flasks.
$MgSO_4.7H_2O$ (Kahlb). Pyrex flasks.

Flasks Reserved for Zinc-free Cultures	Flasks Reserved for Zinc Cultures
* 0.924 g.	0.283 g.
0.336	0.265
0.321	0.313
0.328	0.341
0.355	0.352
0.335	0.311

* An accidental zinc culture. In some cases due to the accidental introduction of zinc, in a few cases to the unintentional use of Jena flasks.

The addition of zinc, etc., where indicated was to the entire solution used in the preparation of cultures having the same concentration of this heavy metal, etc., and not to the individual flasks. Exceptions to this rule, however, were frequently made when only two or three duplicate cultures were prepared. Zinc was always added from one or two stock solutions ($ZnSO_4.7H_2O$, Baker's "Analysed"). One contained 2.5 mg. zinc per cubic centimeter (*i.e.*, 11.0 mg. $ZnSO_4.7H_2O$ per cc.); the other 0.025 mg. zinc per cubic centimeter.

The flasks, each containing 50 cc. of nutrient solution, measured in a 50 cc. graduate, were sterilized at 14½ lbs. for 20 minutes.

Inoculations of the flasks were always from bread cultures in jelly glasses having tinned covers. In order to maintain uniform moisture conditions, the bread (a portion of a slice of fresh bread one inch thick) was supported in the center of the glass by means of a horizontally placed microscope slide so that it did not come in contact with the water in the bottom of the glass. In their preparation the water should be omitted until after sterilization (14½ lbs. for 30 minutes). Immediately after sterilization and while still hot, the proper amount (50 cc.) of sterile water (it should be autoclaved at the same time as are the cultures) was added. In this manner a large surface of growth for the organism was obtained under fairly uniform moisture conditions, and the cultures were ready for inoculation even after several months. Enough spores[1] were added from the bread culture by means of a platinum loop to make a visible and apparently almost continuous layer on the surface of the solution in the flask. Immediately after inoculation the flasks were placed in an incubator (with exclusion of light) at 30–31° C. for seven days.

When harvested, the membrane, together with the solution, was thrown on a washed and weighed filter, washed twice with distilled water and dried at 103–105° C. for four days.

The yields, while given to three places, are probably reliable to the second place only.

It will be noted that the experimental conditions are not entirely uniform. Particularly is this the case with the *A. niger* cultures used. The *A. niger* culture used in these experiments was obtained originally from the "Internationalstelle für Pilz-Kulturen," Amsterdam. Later the two one-spore cultures referred to above as W and Y were isolated from this culture in the manner described in an earlier paper (65).

The two strains thus isolated were kept on agar slants (1 % each of peptone, sucrose, and agar), in the incubator at 30–31° C. Sub-cultures (on bread) for the inoculation of the flasks were also kept in the incubator at 30–31° C. The stock cultures were carried in duplicate, transfers being at irregular intervals. A duplicate stock culture once having been used, for the preparation either of new stock cultures or of a bread culture, was placed aside as unfit for further use. The practise was also followed of preserving a single duplicate stock culture, unused, from each transfer. The numbers given with the designation of the strain refer to the total number of transfers since the original isolation of the strains.

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[1] Approximately four milligrams dry weight.

I present here summaries of three of the 18 experiments presented in the

4. The effect of adding zinc to the culture medium (strain W). Strain W, 6. Each value the average of five duplicate cultures.

Mg. Zn/L	Yield	Sporulation	Mg. Zn/L	Yield	Sporulation
—	0.136 g.	Excellent.	5.0	0.963 g.	Sterile.
0.01	0.287	"	10.0	0.906	"
0.1	0.851	Good.	15.0	0.902	"
0.5	0.843	Moderate.	20.0	0.901	"
1.0	0.915	"	25.0	0.945	"

5. The effect of adding zinc to the culture medium (strain Y). Strain Y, 6. Each value the average of five duplicate cultures.

Mg. Zn/L	Yield	Sporulation	Mg. Zn/L	Yield	Sporulation
—	0.205 g.	Excellent.	5.0	0.757 g.	Good.
0.01	0.283	"	10.0	0.771	"
0.1	0.823	"	15.0	0.746	Fair.
0.5	0.826	Good.	20.0	0.725	"
1.0	0.859	"	25.0	0.754	"

6. The effect of adding zinc to the culture medium (Strains W and Y).

Each value the average of two duplicate cultures, except the controls which are the average of five duplicate cultures.

Strain W, 10

Mg. Zn/L	Yield	Sporulation	Methyl Violet
—	0.177 g.	Excellent.	Blue.
0.01	0.287	"	"
0.02	0.381	"	"
0.03	0.475	"	"
0.04	0.523	"	Green-blue.
0.05	0.534	"	"
0.06	0.688	"	Blue-green.
0.07	0.697	"	"
0.08	0.758	"	Green.
0.09	0.761	"	"
0.10	0.800	"	"

Strain Y, 10

Mg. Zn/L	Yield	Sporulation	Methyl Violet
—	0.222 g.	Excellent.	Blue.
0.01	0.440	"	Green-blue.
0.02	0.501	"	"
0.03	0.563	"	"
0.04	0.642	"	Blue-green.
0.05	0.707	Fair.	Green.
0.06	0.793	"	"
0.07	0.753	"	"
0.08	0.734	Practically sterile.	"
0.09	0.770	" "	"
0.10	0.799	" "	"

paper referred to above (65). We may note that both the zinc optimum and the maximum yield of the two strains differed (experiments 4, 5, and 6).

The above noted cultures indicate also the nature of the results obtained by the addition of zinc sulphate to the Pfeffer nutrient solution. We note that with increasing concentrations of zinc the yield increased and spore formation decreased. The maximum acceleration of growth occurred in the case of both strains in a zinc concentration of 0.1 mg. Zn/L or less, higher concentrations of zinc still resulting in the maximum growth or in a slight decrease. The optimum concentration of zinc for growth as well as the maximum growth obtained is greater for the W than for the Y strain.

Two explanations have been suggested for the method of action of these ions on growth. Javillier (22) is inclined to believe that the increased growth on addition of zinc is due essentially to the increased elaboration of an enzyme—sucrase. The evidence offered does not, however, settle the question as to whether the increased production of sucrase by the organism is the result or the cause of the accelerated growth.

Further there is the suggestion of Loeb (35) that the phenomenon depends on the balancing of the solution. Of this, however, I could find no evidence in my studies. Variation of the concentration and proportions of the constituents of the Pfeffer solution (except iron sulphate) in no case led to the exhibition of phenomena at all comparable to those accompanying the action of zinc. That is, no indication could be obtained that either ammonium, potassium, hydrogen, or magnesium ions were present in toxic concentration. Experiments 7 to 16 inclusive will make this clear.

The Pfeffer nutrient solution contains NH_4NO_3, KH_2PO_4, and $MgSO_4 \cdot 7H_2O$ in amounts of 10 : 5 : 2.5 grams respectively per liter. The compositions of the following solutions have been indicated in the same manner.

7. The effect of a decrease in concentration of the magnesium sulphate.

4 : 2 : 1*	4 : 2 : 0.2	4 : 2 : 0.04	4 : 2 : 0.008
0.139 g.	0.127 g.	0.093 g.	0.038 g.
0.142	0.118	0.074	0.035
0.151	0.110	0.083	0.035
0.131	0.113	0.092	0.032
0.169	0.115	0.091	0.036
0.146	0.117	0.087	0.035

NH_4NO_3		KH_2PO_4		$MgSO_4 \cdot 7H_2O$	Sporulation
4	:	2	:	1	Very good.
4	:	2	:	0.2	Good.
4	:	2	:	0.04	Fair.
4	:	2	:	0.008	Very sparse, very delicate mycelium.

* $MgSO_4 \cdot 7H_2O$ (Kahlbaum).

8. The effect of a decrease in concentration of one or more of the salts of the Pfeffer solution.

10 : 5 : 2.5*	10 : 5 : 1	10 : 2 : 2.5	10 : 2 : 1
† 0.917 g.	0.335 g.	0.343 g.	0.297 g.
0.294	0.311	0.255	0.276
0.303	0.397	0.244	0.301
0.261	0.367	0.271	0.300
0.342	0.415	0.304	0.321
0.300	0.365	0.284	0.299
4 : 5 : 2.5	4 : 5 : 1	4 : 2 : 2.5	4 : 2 : 1
0.254 g.	0.238 g.	0.284 g.	† 0.796 g.
0.207	0.280	0.164	0.179
0.254	0.229	0.162	0.176
0.223	0.243	0.163	0.187
0.152	0.336	0.178	0.189
0.218	0.265	0.190	0.183

* $MgSO_4.7H_2O$ (Kahlbaum).

† Accidental zinc culture.

All fruited in two days.

9. The effect of a decrease in concentration of the monopotassium phosphate.

4 : 2 : 0.01‡	4 : 0.4 : 0.01	4 : 0.08 : 0.01	4 : 0.016 : 0.01
0.128 g.	0.104 g.	0.110 g.	0.097 g.
0.085	0.121	0.158	0.078
0.106	0.138	0.117	0.082
0.245	0.089	0.137	0.077
0.107	0.108	0.119	0.088
0.134	0.112	0.128	0.084

‡ $MgSO_4.7H_2O$ (Kahlbaum).

In these experiments we note that only a decrease in the yield follows a decrease in concentration of one or more of the inorganic constituents of the Pfeffer solution, and we may conclude therefore that none of them are present in toxic or in greater than optimum concentration.

The effects of increasing the concentration of the electrolytes of the Pfeffer nutrient solution as well as of a decrease are shown in experiments 10–16 inclusive.

10. The effect of a variation in concentration of the monopotassium phosphate in the Pfeffer solution.

10 : 25 : 2.5 §	10 : 10 : 2.5	10 : 0.1 : 5	10 : 0.01 : 2.5
0.496 g.	0.335 g.	0.196 g.	0.048 g.
0.389	0.327	0.182	0.048
0.349	0.355	0.136	0.053
0.459	0.334	0.159	0.058
0.556	0.266	0.169	0.065
0.450	0.323	0.168	0.054

§ $MgSO_4.7H_2O$ (Kahlbaum).

In all except 10 : 0.01 : 2.5 (NH_4NO_3 : KH_2PO_4 : $MgSO_4.7H_2O$), good sporulation was obtained; in the latter slightly less sporulation and no membrane, but the solution was full of submerged hyphae, apparently in a solid mass.

11. The effect of a variation in concentration of the ammonium nitrate in the Pfeffer solution.

25 : 5 : 2.5 *	15 : 5 : 2.5	0.1 : 5 : 2.5	0.01 : 5 : 2.5
0.430 g. 0.346 0.403 0.368 0.331	0.409 g. 0.372 0.329 0.324 0.363	0.070 g. 0.058 0.060 0.057 0.061	0.023 g. 0.022 0.029 0.030 0.042
0.376	0.359	0.061	0.029

NH_4NO_3		KH_2PO_4		$MgSO_4.7H_2O$	Sporulation
25	:	5	:	2.5	Good.
15	:	5	:	2.5	"
0.1	:	5	:	2.5	Sparse, solid mass submerged hyphae and membrane.
0.01	:	5	:	2.5	Very sparse, solid mass submerged hyphae and membrane.

* $MgSO_4.7H_2O$ (Kahlbaum).

12. The effect of a variation in concentration of the magnesium sulphate in the Pfeffer solution.

10 : 5 : 25	10 : 5 : 10	10 : 5 : 0.1	10 : 5 : 0.01
0.552 g. 0.450 0.487 0.540 0.488	0.360 g. 0.353 0.387 0.372 0.371	0.156 g. 0.127 0.178 0.182 0.284	0.087 g. 0.087 0.112 0.121 0.118
0.503	0.369	0.185	0.105

Sporulation in all except 10 : 5 : 0.01, good; in latter, fair.

In experiments 10, 11, and 12 we find that if we vary the concentration of ammonium nitrate, of monopotassium phosphate, or of magnesium sulphate in the Pfeffer solution, the others being held constant, the effect of a decreased concentration is a decreased yield, that of an increased concentration an increased yield.

13. The effect of an increase in concentration of the monopotassium phosphate in the Pfeffer solution.

Strain W, 5

Control	KH_2PO_4	Yield
0.133 g.	10 : 29 : 2.5	0.153 g.
0.124	10 : 45 : 2.5	0.246
0.146	10 : 65 : 2.5	0.190
0.140	10 : 85 : 2.5	0.381
0.121	10 : 105 : 2.5	0.369
0.133		

14. The effect of increasing the concentration of the magnesium sulphate in the Pfeffer solution.

$MgSO_4.7H_2O$ (Kahlbaum).

Strain W, 1		Strain Y, 1	
Control	0.01 Mg. Zn/L	Control	0.01 Mg. Zn/L
0.302 g.	0.602 g.	0.516 g.	0.806 g.
0.265	0.578	0.535	0.830
0.339	0.562	0.546	0.843
0.330	0.528	0.409	0.694
0.335	0.554	0.393	0.732
0.317	0.565	0.480	0.781

Strain W, 1

10 : 5 : 22.5	10 : 5 : 42.5	10 : 5 : 62.5	10 : 5 : 82.5	10 : 5 : 102.5
0.530 g.	0.586 g.	0.669 g.	0.621 g.	0.810 g.
0.372	0.448	0.638	0.730	0.907
0.451	0.517	0.654	0.676	0.859

Strain Y, 1

10 : 5 : 22.5	10 : 5 : 42.5	10 : 5 : 62.5	10 : 5 : 82.5	10 : 5 : 102.5
0.600 g.	0.663 g.	0.817 g.	0.746 g.	0.980 g.
0.487	0.600	0.757	0.775	1.017
0.544	0.632	0.787	0.761	0.999

Retardation of spore formation only in 10 : 5 : 102.5 (NH_4NO_3 : KH_2PO_4 : $MgSO_4.7H_2O$). With increased concentrations of $MgSO_4.7H_2O$ the spores became more and more brown colored.

15. The effect of increasing the concentration of the magnesium sulphate in the Pfeffer solution.

Strain W, 2

10 : 5 : 2.5	10 : 5 : 77.5	10 : 5 : 83.75	10 : 5 : 90	10 : 5 : 96.25
0.224 g.	0.613 g.	0.646 g.	0.695 g.	0.735 g.
0.248	0.625	0.674	0.745	0.800
0.236	0.619	0.660	0.720	0.768

10 : 5 : 102.5	10 : 5 : 108.75	10 : 5 : 115.0	10 : 5 : 121.25	10 : 5 : 127.5
0.722 g.	0.832 g.	0.865 g.	0.818 g.	0.929 g.
0.747	0.778	0.802	0.834	0.930
0.735	0.810	0.834	0.824	0.930

Strain Y, 2

10 : 5 : 2.5	10 : 5 : 77.5	10 : 5 : 83.75	10 : 5 : 90.0	10 : 5 : 96.25
* 0.720 g.	0.661 g.	0.647 g.	0.743 g.	0.658 g.
0.511	0.720	0.750	0.692	0.804
0.511	0.691	0.699	0.718	0.731

* Accidental zinc culture.

10 : 5 : 102.5	10 : 5 : 108.75	10 : 5 : 115.0	10 : 5 : 121.25	10 : 5 : 127.5
0.787 g. 0.685	0.794 g. 0.819	0.863 g. 0.767	0.829 g. 0.854	0.887 g. 0.824
0.736	0.807	0.815	0.842	0.856

Fructification initiated in all cultures (except the accidental zinc) at about the same time—second day. Spores in all cultures brown, except that the membranes of all but the two magnesium sulphate concentrations (77.5 and 83.75) of the W strain had a ring of jet black spores on the margins.

16. The effect of increasing the concentration of the magnesium sulphate in the Pfeffer solution.

Strain W, 3		Strain Y, 3	
10 : 5 : 2.5	10 : 5 : 130	10 : 5 : 2.5	10 : 5 : 130
* 0.908 g. 0.127 0.078 0.081 0.133	0.899 g. 0.959 0.859 0.895 0.827	0.327 g. 0.362 0.341 0.206 0.268	0.756 g. 0.851 0.924 0.784 0.725
0.105	0.888	0.301	0.808

Complete sporulation took place in the W strain in 2½ days; in the Y strain in 2 days.

* Accidental zinc culture.

Here again (experiments 7 to 16) we note only a decrease in growth resultant upon decreasing the concentration of one or more of any of the three components of the Pfeffer nutrient solution. As a result of their increase we obtain, however, an increased growth. In the case of NH_4NO_3 and KH_2PO_3 the acceleration of growth is but a moderate one in spite of the fact that these compounds were added in so high amounts that the action of the impurities they doubtless contained might have been manifested. Only magnesium sulphate, it will be noted, gave results at all comparable to those with zinc, and then only in very high concentration. The high concentrations used renders it very probable that the action of the magnesium sulphate is to be attributed to the presence of zinc, etc., in traces. Since we have seen in previous experiments that the zinc optimum is approximately 0.1 mg. Zn/L, we can calculate that in a ten percent solution of magnesium sulphate zinc need only be present in 0.0001 percent impurity to be thus added in sufficient amount to cause maximum acceleration of growth. The results obtained with magnesium sulphate are discussed more in detail later. The evidence is quite conclusive, however, that neither the ammonium nitrate, the potassium phosphate, nor the magnesium sulphate is present in greater than optimum or in toxic concentration.

On inspection of the data (see table below) as to the ions whose presence results in an acceleration of the growth of *A. niger*, it is obvious that the majority are salts of heavy metals. The salts of the heavy metals, when the anion is that of a strong acid, hydrolyze in aqueous solution, resulting, as a rule, in increased acidity of the solution. Of the seventeen ions ($[H^+]$ is included) contained in this list, thirteen are reported as acting in this manner. The apparent exception in the case of the ferrous and manganous ions disappears on recognition of the ease with which their solutions are oxidized when in contact with the atmosphere. Ferric and manganic (14) ions behave similarly to the other heavy metal ions, as is generally reported.

In the case of only two of the ions listed above (F^- and $SiO^{-}{}_{3}{}^{-}$) does the hydrolysis of the majority of their salts lead to decreased acidity of the solution. In the case of the fluorides, the ammonium salt (NH_4F) from what can be gathered from data at our disposal, appears to hydrolyze in aqueous solution (14) with increased acidity due to the formation of HNH_4F_2.

A. niger

Compounds Reported as "Stimulants"

Compound	Max. Yield / Control	Optimum of "Stimulant"	Author	% Hydrolysis at 25° C. (27)	Solution Acid to Litmus (40)
$ZnSO_4$......	4.69 /1.55	0.1 mgZn/L	Javillier (21)	m/5 = 0.0075	+
$MnSO_4$.....	0.982/0.610	10 g.Mn/L	B. & J. (4)	None?	o
$CdSO_4$.....	0.750/0.277	0.01 mgCd/L	Lepierre (29)	m/5 = 0.017	+(*)
$BeSO_4$......	0.490/0.281	0.01 mgBe/L	" (30)		+(66)
$UO_2(NO_3)_2$.			" (31)		+
$HgCl_2$......	0.630/0.341	0.0013%	Ono (49)	m/256 = 1.43	+
$MnCl_4$......	0.380/0.245	0.004%	Richards (57)		+
$CoSO_4$......	0.805/0.550	0.002%	"	m/32 = 0.015	+(*)
$CoSO_4$......	0.872/0.297	0.014%	Ono (49)		+
$NiSO_4$......	0.785/0.200	0.033%	Richards (57)	m/32 = 0.044	+(13)
$NiSO_4$......	0.404/0.262	0.014%	Ono (49)		+
$FeSO_4$......	0.892/0.275	0.132%	Richards (57)	None?	o
$CuSO_4$	0.352/0.218	0.003%	Ono (49)	m/5 = 0.05	+
NaF.......	0.640/0.330	0.002%	Richards (57)		−(66)
NaF.......	0.325/0.199	0.0025%	Ono (49)		−
LiCl.......	0.410/0.280	0.016%	Richards (57)		?
Li_2SO_4......	0.560/0.235	0.5%	"		?
$LiNO_3$......	0.428/0.300	0.008%	"		+(*)
Na_2SiO_3....	0.575/0.350	0.004%	"		−(66)
$Al_2(SO_4)_3$...	0.260/0.205	0.002%	"		+
$Fe_2(SO_4)_3$...	0.6+/0.1+		Steinberg		+
Acids......	0.3 /0.13		"		+

* Tested the Kahlbaum compounds personally. In the case of the lithium salts only the nitrate was available.

I have repeated Richards' (57) experiments with sodium silicate. The results of my experiments are negative. They are tabulated below (experiments 17 and 18).

17. The effect of adding sodium silicate to the culture medium.

Strain W, 11

Control	Na_2SiO_3 (Kahlbaum)	
0.066 g.	0.002%	0.073 g.
0.035	0.008	0.073
0.086	0.098	0.078
0.084		
0.068		

There appeared no difference with respect to sporulation between the controls and the sodium silicate cultures. The acidities of all cultures at the time of harvest corresponded to $p_H = 3–4$.

18. The effect of adding sodium silicate to the culture medium.

Strain W, 11			
Na_2SiO_3 (Kahlb.)	Yield	Na_2SiO_3 (Kahlb.)	Yield
0.%	0.176 g.	0.12%	0.236 g.
0.002	0.195	0.18	0.139
0.01	0.154	0.26	0.126
0.05	0.230	0.32	0.144
0.08	0.207	0.40	0.162

No differences in spore formation appeared. Acidities at the time of harvest were equivalent to $p_H = 3 - 4$.

My cultures showed none of the ordinary "stimulation" effects, and I am inclined to associate these results with the fact that the addition of the silicate does not result in the increased acidity of the nutrient solution.

Further exceptions to this general rule that "stimulants" are electrolytes whose cations form weakly ionized bases of low solubility (*i.e.*, dissociate hydrolytically in the presence of an anion of a strong acid so as to cause an increase in acidity) are found possibly in experiments by Richards (57) and Yasuda (69) indicating that alkaloids, and by Miss Latham (28) indicating that chloroform, may cause growth "stimulation."

Finally there remain the substances used by Schulz (62) in his studies on acceleration of respiration with yeast: $HgCl_2$, I, KI, CrO_3, sodium salicylate, formic acid, and arsenious acid; and those used by Fred (16) in his studies on bacteria: ether, carbon disulphide, potassium dichromate, $CuSO_4$, and salvarsan. Of these thirteen substances, six agree in their ability to cause an increase in acidity; two must be grouped with chloroform as comparatively chemically inert, and the position of the others: I (and KI), Br, sodium salicylate, and salvarsan are doubtful.

It is to be noted for all these cases that practically the difficulty is not to induce the appearance of the phenomena associated with zinc "stimulation," but to prevent their occurrence. Substances exercising an action analogous to that of zinc may very easily be accidentally introduced through use of improper glassware (64) and impure chemicals. Particularly is one to exercise great caution in the interpretation of results obtained with compounds in high concentration, since any impurity they contain may thus be presented to the organism in effective concentration. An impurity of one-thousandth of one percent may cause the introduction in five percent solution of a compound of 0.5 mg./L impurity.

Further I have no wish to suggest that the degrees of growth "stimulation" are always the result of varying acidities. Without doubt, it is possible that even if the majority of these substances actually function through their effect on the acidity of the nutrient solution, others by influencing the organic processes at another stage, or in another way, may bring about the same result.

The effect of increased acidity, and of decreased acidity as well, of the Pfeffer nutrient solution are indicated in the following experiments. Increased acidity of the nutrient solution was brought about through the addition of different free concentrated acids immediately after sterilization. In order to obtain cultures of lower acidity than the Pfeffer solution, free alkali was added. In all cases full precautions were taken for the exclusion of zinc, etc.

19. The effect of increasing the acidity of the culture medium.

Strain W, 5

Control	H_3PO_4 (Kahlb. kryst.)	
0.133 g.	0.1 cc./flask	0.279 g.
0.124	0.2	0.302
0.146	0.3	0.307
0.140	0.4	0.232
0.121	0.5	0.178
0.133		

20. The effect of an increase or of a decrease in acidity of the culture medium.

Strain W, 6

Control	KOH (5:6)*	
0.107 g.	0.1 cc./flask	0.109 g.
0.126	0.2	0.055
0.126	0.3–0.5	No growth
0.120		

NH_4OH (Sp. Gr. 0.90)		HNO_3 (Sp. Gr. 1.42)	
0.1 cc./flask	0.065 g.	0.1 cc./flask	0.200 g.
0.2	0.030	0.2–0.5	No growth.
0.3	0.023		
0.4–0.5	No growth.		
HCl (Sp. Gr. 1.18)		H_2SO_4 (Sp. Gr. 1.84)	
0.1 cc./flask	0.291 g.	0.1–0.5 cc./flask	No growth.
0.2	0.129		
0.3	0.106		
0.4–0.5	No growth		

The hyphae in the cultures to which KOH or NH_4OH was added were watery and translucent in appearance. Sporulation in these cultures was very good. In those to which HNO_3 or HCl was added the hyphae were opaque, the membranes wrinkled, and spore formation was retarded.

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* Baker's "Analysed": NH_4OH, KOH, HNO_3, HCl, $Ba(OH)_2$, tartaric acid. Baker's "U.S.P.": acetic acid. Baker's "special": oxalic acid, H_2SO_4. Kahlbaum: H_3PO_4 kryst.

21. The effect of an increase or of a decrease in acidity of the culture medium.

Strain W, 7				
Control	NH_4OH (Sp. Gr. 0.90)		KOH (5 : 6)	
*0.907	0.02 cc./flask	0.214 g.	0.02 cc./flask	0.141 g.
0.211	0.04	0.135	0.04	0.140
0.196	0.06	0.158	0.06	0.185
0.112	0.08	0.224	0.08	0.182
0.112	0.10	0.131	0.10	0.183
0.126				

HNO_3 (Sp. Gr. 1.42)		HCl (Sp. Gr. 1.18)		H_2SO_4 (Sp. Gr. 1.84)	
0.02 cc./flask	0.130 g.	0.02 cc./flask	0.112 g.	0.02 cc./flask	0.244 g.
0.04	0.233	0.04	0.115	0.04	0.336
0.06	0.282	0.06	0.211	0.06	0.239
0.08	0.289	0.08	0.329	0.08	0.110
0.10	0.266	0.10	0.274	0.10	0.028

* Accidentally used a Jena flask.

I accidentally added the NH_4OH, KOH, HNO_3, etc., to the flasks before sterilization. Spore formation in all except the cultures to which acids were added was excellent. Good sporulation in all cultures with HNO_3 and HCl. H_2SO_4 : 0.02 cc., excellent spore formation, translucent hyphae; 0.04 cc., opaque hyphae; 0.06 cc., dense white hyphae, slight retardation of spore formation; 0.08 cc., thin, dense white membrane, few spores; 0.10 cc., submerged hyphae, no membrane.

22. The effect of an increase in acidity of the culture medium.

Strain W, 7				
Control	Tartaric Acid		Acetic Acid (99%)	
0.078 g.	0.5 cc./flask	0.184 g.	0.1 cc./flask	0.236 g.
0.082	1.0	0.305	0.2–0.5	No growth
0.091	1.5	0.352		
0.087	2.0	0.387		
0.090	2.5	0.407		
0.086				

A time retardation of spore formation was evident in cultures containing 2.0 and 2.5 g. tartaric acid to the 50 cc. of nutrient solution.

23. The effect of an increase or of a decrease in acidity of the culture medium.

Strain W, 8				
Control	NH_4OH (Sp. Gr. 0.90)		KOH (5 : 6)	
0.131 g.	0.05 cc./flask	0.166 g.	0.02 cc./flask	0.107 g.
0.135	0.1	0.055	0.05	0.081
0.112	0.2	0.039	0.1	0.057
0.111	0.3	No growth	0.2	0.075
0.136	0.4	“ “	0.3	No growth
0.125				

$Ba(OH)_2$ (0.084 N)		HNO_3 (Sp. Gr. 1.42)	
0.1 cc./flask	0.117 g.	0.02 cc./flask	0.070 g.
0.3	0.101	0.05	0.125
0.5	0.157	0.08	0.169
1.0	0.180	0.1	0.336
1.5	0.163	0.2	No growth

H_3PO_4 (Kahlb. Kryst.)	
0.1 cc./flask	0.303 g.
0.2	0.286
0.3	*0.905
0.4	0.266
0.5	0.180

H_2SO_4 (Sp. Gr. 1.84)		HCl (Sp. Gr. 1.18)	
0.02 cc./flask	0.188 g.	0.05 cc./flask	0.107 g.
0.04	0.244	0.1	0.261
0.06	0.260	0.2	0.227
0.08	0.167	0.3	0.039
0.10	0.034	0.4	No growth

$HC_2H_3O_2$ (99%)	
0.02 cc./flask	0.225 g.
0.05	0.253
0.08	0.279
0.1	0.280
0.2	No growth

$H_2C_2O_4$		+0.1 Mg. Zn/L	
0.1 g./flask	0.202 g.	0.08 cc. H_2SO_4/flask	0.572 g.
0.2	0.289		
0.3	0.337	0.02 cc. NH_4OH/flask	0.203
0.4	0.233		
0.5	0.043		

The reactions of the freshly prepared cultures were all acid to litmus except: KOH—0.2 and 0.3 cc.; and NH_4OH—0.2, 0.3, and 0.4 cc.

On the second day sporulation was complete in the controls: NH_4OH—0.05 and 0.1 cc.; $Ba(OH)_2$—all; $HC_2H_3O_2$—0.02, 0.05, and 0.08 cc. On the seventh day sporulation was completed in the controls, and in the cultures to which KOH, NH_4OH, $Ba(OH)_2$, and $HC_2H_3O_2$ had been added. The lowest concentrations of acid causing partial suppression of spore formation were: HNO_3—0.1 cc.; H_3PO_4—0.4 cc.; H_2SO_4—0.06 cc.; HCl—0.2 cc. and $H_2C_2O_4$—0.2 g.

As respects the cultures to which zinc had been added, those containing H_2SO_4 was practically sterile, those containing NH_4OH black with spores.

* Accidental zinc culture.

24. The effect of an increase or of a decrease in acidity of the culture medium.

Strain W, 11			
Control	0.1 Mg. Zn/L	0.2 Cc. NH_4OH/Flask	0.2 Cc. H_3PO_4/Flask
0.066 g.	0.869 g.	0.016 g.	0.229 g.
0.035	0.853	———‡	0.233
0.086	0.862	0.026	0.226
0.084	0.782	0.024	0.262
† 0.677	0.911	———	* 0.902
0.068	0.855	0.022	0.238

Acidities on harvest corresponded to $p_H = 3–4$ (control, NH_4OH); 2–3 (H_3PO_4), and 1–2 (0.1 mg. Zn/L).

* Accidental zinc culture.

† Accidental stimulation resulting from addition of methyl violet 6 B extra Grübler, which may or may not be due to impurities.

‡ Not inoculated: p_H on harvest < 6.8; of the inoculated flasks $p_H = 3–4$.

The addition of acid to the Pfeffer nutrient solution resulted (experiments 19 to 24 inclusive) in an acceleration of growth. The maximum acceleration of growth, no matter which of the acids was used, was but slightly more than double that of the control. The effect of the addition of alkali to the Pfeffer solution was a diminution of the yield. Furthermore, the effect of the increased acidities of the cultures was to cause a retardation or suppression of spore formation as distinguished from the effects of decreased acidity which did not cause the exhibition of the same phenomenon.

All seven acids used in the above described experiments gave results essentially similar; an indication that these results are due to the hydrogen ion primarily and not to the anions. The acceleration of growth due to tartaric acid, however, needs to be verified, since I am inclined to attribute its action, at least in the sample used, to the presence of impurities. As a result of increased acidity, it will be noted, the yield can be doubled; while a decrease in the acidity of the Pfeffer solution acts in an opposite manner. My experiments serve, therefore, to confirm the long recognized fact that a fluctuation in the acidity brings about a corresponding variation in the yields; and moreover, that the acidity of the Pfeffer solution is sub-optimal for the growth of *A. niger*.

I have studied the appearance of the mycelia and of the modification of sporulation in cultures on Pfeffer solution of varying acidity. In all respects the agreement between the effects of increased acidity and the effects of ions like zinc and iron appears to be complete. The effects of decreased acidity and of increased acidity of the Pfeffer nutrient solution are distinct and opposite. Excessive acidity or alkalinity may, it is true, entirely prevent growth. Within these limits, however, an acidity lower than that of the Pfeffer solution ($p_H = 3$–4) results in a decreased yield, the hyphae are dark and watery in appearance—translucent—and the thin, flat membranes are black with spores. Increased acidity causes increased growth, the hyphae are white or light tan in color and heavy-walled—opaque—and the thickened and wrinkled membranes may show a retardation of spore formation.

The appearance and yields of the cultures in Pfeffer solution of higher acidity (greater than $p_H = 3$–4) are entirely similar to those containing zinc and iron. Cultures of lower acidity than that of the Pfeffer solution are similar to those from which zinc, etc., ions have been excluded. Moreover, a regular transition in the appearance and yields of the cultures is apparent from those of maximum alkalinity to those of maximum acidity.

The phenomena of chemical "stimulation" of *A. niger*: (*a*) increased growth, (*b*) increased respiration, (*c*) increased fermentative activity, (*d*) modification of the character of the cell walls, and (*e*) retardation or suppression of sporulation, are quite the same as those associated with the action of the hydrogen ion on the fungus.

Assuming that the H-ion concentration is a source of "stimulation," it should of necessity follow that a modification of the hydrogen-ion concentration of the Pfeffer nutrient solution would result in the modification of the optimum concentration of other "stimulants" for growth, and in such a sense that increased acidity brings about a decrease in the optimum of the "stimulant" and a decrease in the acidity an increase in the optimum of the "stimulant." Some evidence bearing on this question is furnished in the experiments of Richards (57), who has already pointed out that in the presence of the optimum iron concentration, the addition of zinc causes no further increase in growth. In the case of manganese and zinc, Bertrand and Javillier (5) found that even a further increase in yield took place in optimum concentration of either if the other was added. This modification of the optimum is easily demonstrated, as reference to the following experiments (25, 26) will show. See also experiment 23.

25. The effect of an increase or of a decrease in acidity of the culture medium on the growth accelerations obtained by addition of zinc.

Strain W, 8

——	+ 0.2 cc. NH_4OH	+ 0.2 cc. H_3PO_4
0.136 g.	0.015 g.	0.329 g.
0.200	0.020	0.313
0.139	0.025	0.334
0.135	0.018	0.318
0.095	0.024	0.299
0.141	0.020	0.319

Mg. Zn/L	——	+ 0.2 Cc. NH_4OH	+ 0.2 Cc. H_3PO_4
0.025 g.	0.358 g.	0.060 g.	0.614 g.
0.05	0.519	0.111	0.787
0.1	0.820	0.241	0.879
1.0	0.929	0.651	0.903
10.0	0.945	0.661	0.866
25.0	0.950	0.767	0.866
50.0	0.957	0.704	0.852
100.0	0.972	0.598	0.605

Acidities of the Culture Nutrient Solutions at Time of Harvest

Mg. Zn/L	——	+ 4 Cc. NH_4OH/L	+ 4 Cc. H_3PO_4/L
—	p_H = 3–4	p_H = 3–4	p_H = 2–3
0.025	≦ 3	≦ 3	2–3
0.05	2–3	≦ 3	2–3
0.1	1–2	2–3	2
1.0	1–2	2–3	1–2
10.0	1–2	2–3	1–2
25.0	1–2	2–3	1–2
50.0	1–2	≦ 3	1–2
100.0	1–2	≦ 3	1–2

The curves obtained by plotting yield against zinc concentration make somewhat more evident the variation of the zinc optimum as a result of the modification in acidity of the Pfeffer nutrient solution. Figure 1, including all the zinc concentrations used, is given for its general interest in depicting the types of curves obtained.

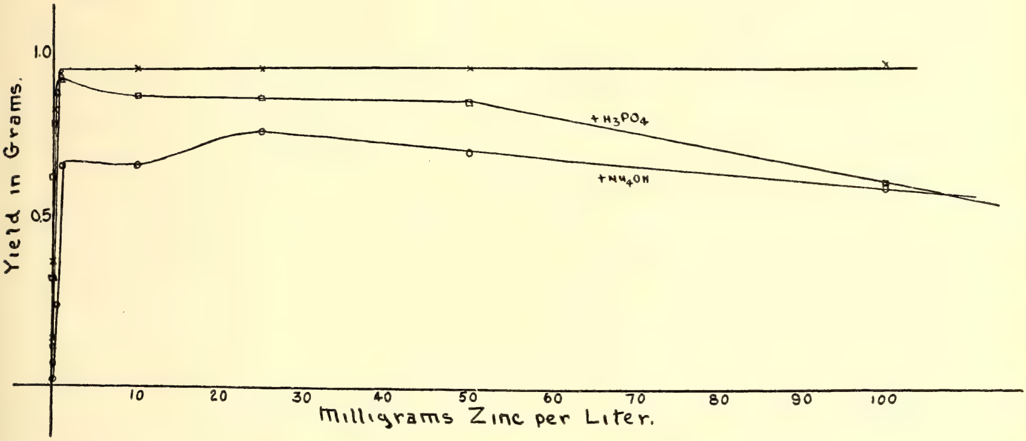

FIG. 1. For explanation see text.

Figure 2 shows more clearly the portions of these curves concerned with sub-optimal values.

We see that for any particular zinc concentration up to about 0.6 mg. Zn/L the amount of growth is least in the presence of NH_4OH and greatest in the presence of H_3PO_4. The zinc optimum also, as closely as these

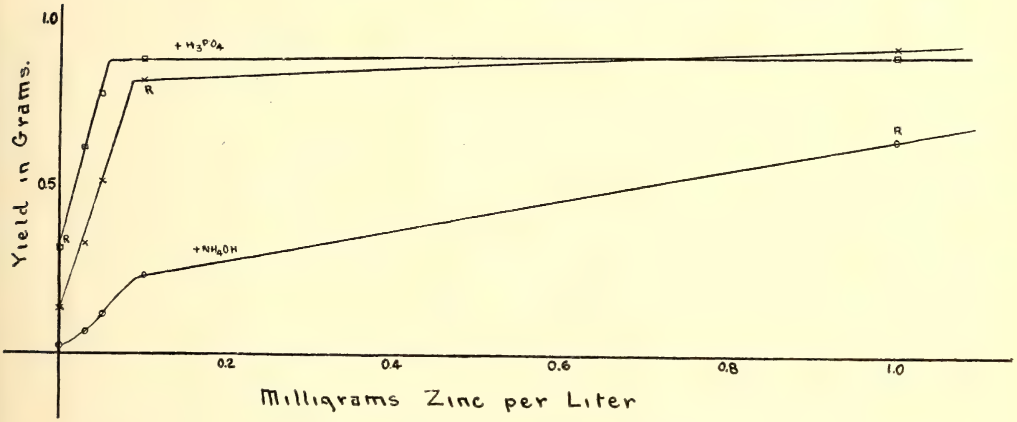

FIG. 2. For explanation see text.

values permit of the location in each case, is about 0.06 mg. Zn/L in the presence of H_3PO_4; about 0.08 mg. Zn/L in the Pfeffer solution without any addition; while 0.1 mg. Zn/L may be taken as the optimum in the presence of NH_4OH. The value 0.08 mg. Zn/L for the nutrient solution without addition of either phosphoric acid or ammonium hydroxide compares well with the value (0.09 mg. Zn/L) found in another experiment (65). The curve obtained in the presence of NH_4OH is rather peculiar, and it may well be that the optimum should be taken in this case as in the neighborhood of 25 mg. Zn/L.

Increased acidity and presence of zinc ions exercise the same effect on the growth of *A. niger*—a positive acceleration. The more zinc the lower the acidity (and vice versa) required for the production of the maximum yield. The data already given show this to be the case. I am inclined to infer, although the authors do not themselves suggest this interpretation, that the same relation appears to hold for the action of iron and increased acidity on the rice plant (Gile and Carrero, 18, p. 521, tables 13 and 14); and for the action of aluminum and increased acidity (barley and rye) studied by Hartwell and Pember (19).

Not only the yields but also the fructification and the acidity attained in the cultures are correlated with the increased growth. In Figure 2 I have indicated by the letter "R" the lowest zinc concentrations exercising a perceptible effect on the sporulation of the cultures. These values are also contained in the following table (part of experiment 25). The hydrogen ion exponent (p_H) of the solutions at the time of harvest has also been added (see below).

Spore Formation in Pfeffer Solution

Partially retarded	
+ 0.2 cc. NH_4OH/flask + 1 mg. Zn/L	p_H = 2–3
+ 0.1 mg. Zn/L	1–2
+ 0.2 cc. H_3PO_4/flask	2–3
Sterile, etc.	
+ 0.2 cc. NH_4OH/flask + 100 mg. Zn/L (vigorous)	$p_H \leqq 3$
+ 100 mg. Zn/L (sparse)	1–2
+ 0.2 cc. H_3PO_4/flask + 1.0 mg. Zn/L (sterile)	1–2

Sporulation is therefore retarded through increased acidity and within limits favored by a decreased acidity. The first indication of retardation of spore formation occurs at an acidity corresponding to a hydrogen exponent of 2–3. In the ammonium hydroxide series spore formation was vigorous even in the presence of 100 mg. Zn/L. Here the low acidity ($p_H \leqq 3$) is without doubt the correct explanation. The extent of the retardation of sporulation depends apparently on the acidity attained in the culture through the growth of the organism.

Use was made of the indicator method as given by Michaelis (41) for the estimation of the above listed acidities. The following results (part of experiment 25) were also obtained in this manner.

It will be noted that the cultures as a result of the growth of the organism attain an increased acidity in all cases, except in the Pfeffer solution without any additions and apparently in the Pfeffer solution to which phosphoric acid was added.

Pfeffer Nutrient Solution	Before Inoculation	At Time of Harvest
—	p_H = 3–4	p^H = 3–4
+ 0.1 mg. Zn/L	3–4	1–2
+ 1.0 "	3–4	1–2
+ 10.0 "	3–4	1–2
+ 0.2 cc. NH_4OH/flask	< 6–8	3–4
+ 0.2 cc. H_3PO_4/flask	2–3	2–3

Additional evidence of the increased acidity attained through the growth of the organism and its relation to the zinc content of the cultures is given in experiment 25 (see above). See also experiment 6, etc.

These acidity values though quite crude serve nevertheless to indicate clearly the existence of a parallelism between increase in growth, retardation of fructification, and increase in acidity of the medium. The maximum acidity attained is $p_H = 1–2$ and corresponds (though see H_3PO_4 series) to maximum growth. "Stimulation" due to iron salts (ferric and ferrous phosphate and ferric sulphate) results in the same acidity. This value, it is to be seen, is in agreement with that of Clark and Lubs (10) — $p_H = 1.7$, and that of Currie (11)—$p_H = 1.46$.

A few values obtained with the "Y" strain (experiment 26) are of essentially the same significance as those with the "W" strain (experiment 25).

26. The effect of an increase or of a decrease in acidity of the culture medium on the growth accelerations obtained by addition of zinc.

Strain Y, 8

Control	Yield	p_H on Harvest	Fructification
+ 0.2 cc. NH_4OH/flask	0.233 g.	2–3	Black with spores
+ 0.2 cc. H_3PO_4/flask	0.070	$\leqq 3$	Retarded
+ 0.2 cc. NH_4OH/flask + 0.1 mg. Zn/L	0.420	2–3	Sterile
" " " + 1.0 " "	0.647	$\leqq 3$	"
" " " + 10.0 " "	0.651	2–3	"
	0.595	2–3	"

I append also an experiment (experiment 27) in which acid variation was estimated by titration. Ten cubic centimeters of each culture was titrated with sodium hydroxide in the presence of methyl orange.

27. The effect of the addition of zinc to the culture medium on the rate of increase and the total acidities developed in the cultures.

Strain W, 11

Harvested	Yields		N/10 NaOH	
	No Zinc	0.15 Mg. Zn/L	No Zinc	0.15 Mg. Zn/L
1 day	0.084 g.	0.096 g.	0 cc.	0.53 cc.
2 days	0.117	0.297	0.86	2.80
3 "	0.088	0.507	2.45	5.25
4 "	0.149	0.670	1.23	5.95
5 "	0.230	0.781	2.10	7.00
7 "	0.176	0.910	1.93	7.88

Here we note that the total free acid in the culture solutions shows a regular and marked increase in total acidity only in the case of the zinc cultures.

It is of course always to be remembered that in order to get an acidity sufficient to be of even a barely perceptible physiological significance as a "stimulant," there must be a concentration of the salt of the heavy metal which is very high from the standpoint of its direct physiologically "stimulative effect." This is of course a strong argument against the assumption that the effect produced by "stimulants" is due to the increased acidity of the solution resulting from their hydrolytic dissociation. Nevertheless, I am inclined, on the basis of the very positive evidence that an increase in the acidity of the Pfeffer solution results in a marked "stimulation" of growth, to ascribe an important physiological effect to the increased H-ion concentration resulting directly from the dissociation of these salts in the culture medium. It is perhaps not impossible that in case the "stimulants" are adsorbed we may get a membrane concentration which would bring the acidity more nearly within the range of strengths necessary to produce directly "stimulative effects" on growth.

Thus it has been shown by Denham (13) that the hydrolysis of salts of this type can result in hydrogen-ion concentrations of considerable magnitude. It will suffice to take the values found in n/16 solution. This concentration is of course much greater than that often employed in the cultures.

Salt (n/16)	$[H^+]$	P_H(calc.)
Tl_2SO_4	0.609×10^{-2}	2.215
$CrCl_3$	0.234×10^{-2}	2.631
Al_2Cl_6	0.152×10^{-2}	2.818
$Al_2(SO_4)_3$	0.145×10^{-2}	2.839
$C_6H_5NH_3HCl$	0.114×10^{-2}	2.944
$NiSO_4$	0.440×10^{-4}	4.357
$CoSO_4$	0.107×10^{-4}	4.971
NH_4Cl	0.427×10^{-6}	6.370

The number of salts experimented with in this connection is so few that it is unfortunately impossible to ascertain whether any relation exists between p_H and the optima for, or the magnitude of the acceleration of, growth. Nevertheless, it is evident that their action does not depend entirely on the acidity attained through hydrolysis, since trivalent aluminum has comparatively little "stimulative effect" in comparison with bivalent cobalt and nickel. Further, the increased yield due to increased acidity is much less than that brought about by the addition of zinc and iron. The addition of the majority results, however, in growth accelerations of the same magnitude as that caused by increased acidity. This variation in effectiveness of the different "stimulants" from the point of view of increased acidity may be accounted for on the basis of impurities, valence, differences in ease of adsorption, degree of hydrolytic dissociation, or by the existence of a distinct action of the "stimulative" ion apart from its effecting an increase in acidity.

Another aspect of the difference in growth acceleration attained by increased acidity and by addition of the zinc ion lies in the probability that there is a variation in optimum acidity for *A. niger* at different stages of development (9). The growth in the Pfeffer solution (p_H = 3–4), I have repeatedly noticed, is greater than in the same solution to which phosphoric acid is added (0.2 cc.) for the first twenty-four hours. Nevertheless, the growth is as one to two at the end of seven days. The initial retardation is more than compensated for later. Experiments were therefore performed with the idea of ascertaining if a still greater growth acceleration with the hydrogen ion could be obtained through the gradual addition of free acid from day to day in comparison with that previously obtained by adding acid only before inoculation (experiments 28, 29, 30, 31).

All the culture flasks in experiments 28, 29, 30, and 31 were provided with a short glass tube inserted through the cotton plug of the flask so that the lower end almost reached to the bottom of the flask, while the upper end (plugged with cotton) projected above the cotton plug of the flaks.

28. The effect of a daily increase in acidity of the culture medium on the growth.

H_3PO_4/Flask				Strain W, 11
Initial	1 Day	2 Days	Total	Yield
0 cc.	0 cc.	0 cc.	0 cc.	0.156 g.
0	0.1	0.1	0.2	0.460
0.05	0.1	0.1	0.25	0.558
0.1	0.1	0.1	0.3	0.318

29. The effect of a daily increase in acidity of the culture medium on the growth.

Strain W, 11						
H_3PO_4/Flask						
Initial	First Day	2	3	4	Total	Yield
0 cc.	0 cc.	0 cc.	0 cc.	0 cc.	0 cc.	0.203 g.
0.2	0	0	0	0	0.2	0.374
0.3	0	0	0	0	0.3	0.443
0.4	0	0	0	0	0.4	0.400
0	0.05	0.1	0.1	0	0.25	0.328
0	0.05	0.1	0.1	0.1	0.35	0.564
0.05	0.1	0.1	0	0	0.25	0.398
0.05	0.1	0.1	0.1	0	0.35	0.371
0.1	0.1	0.1	0	0	0.3	0.520
0.1	0.1	0.1	0.1	0	0.4	0.504

+ 0.15 mg. Zn/L 0.910 g.

30. The comparative yield resulting from the addition of varying amounts of acid before inoculation and on the first day after inoculation.

Strain W, 11			
H_3PO_4/Flask		H_3PO_4/Flask	
Added Before Inoculation	Yield	1 Day	Yield
0 cc.	0.204 g.	0.1 cc.	0.332 g.
0.2	0.327	0.2	0.267
0.3	0.638	0.3	0.435
0.4	0.544	0.4	0.464
0.5	0.246	0.5	0.285
0.6	0.166	0.6	0.202
0.7	0.049	0.7	0.215
0.8	0.040	0.8	0.186
H_3PO_4/Flask			
Initial	1st Day	2	Yield
0.1 cc.	0.3 cc.	0.1 cc.	0.232 g.
0.1	0.3	0.1	0.302
0.1	0.2	0.2	0.199
0.1	0.2	0.2	0.351

0.2 mg. Zn/L 0.985 g

31. The comparative yield resulting from the addition of varying amounts of acid before inoculation and on the first, second, and third days after inoculation.

Strain W, 11					
H_3PO_4/Flask Added Before Inoculation		H_3/PO_4Flask Added 24 Hrs. After Inoculation		H_3PO_4/Flask Added 48 Hrs. After Inoculation	
0 cc.	0.183 g.	0.2 cc.	0.290 g.	0.2 cc.	0.241 g.
0.2	0.402	0.3	0.323	0.3	0.300
0.3	0.377	0.4	0.257	0.4	0.299
0.4	0.347	0.5	0.265	0.5	0.279
0.5	0.182	0.6	0.255	0.6	0.247
0.6	0.078				

H_3PO_4/Flask Added 72 Hrs. After Inoculation		Mg. Zn/L	Yield
0.2 cc.	0.244 g.	0	0.123 g.
0.3	0.325	0.025	0.339
0.4	0.274	0.05	0.378
0.5	0.310	0.075	0.447
0.6	0.215	0.1	0.540
		0.125	0.724
		0.15	0.755
		0.175	0.872
		0.2	0.909

The cultures of the zinc series were prepared as usual, *i.e.*, there was no tube penetrating through the cotton plug of the flask.

(*Concluded in the November number.*)

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Vol. VI. NOVEMBER, 1919 No. 9

AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

DEC 19 1919

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PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT 41 NORTH QUEEN STREET, LANCASTER, PA.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania, under the act of March 3, 1879

AMERICAN JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE BOTANICAL SOCIETY OF AMERICA

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AMERICAN
JOURNAL OF BOTANY

Vol. VI November, 1919 No. 9

A STUDY OF SOME FACTORS IN THE CHEMICAL STIMULATION OF THE GROWTH OF ASPERGILLUS NIGER

Robert Aaron Steinberg

(Concluded from the October Journal)

The results obtained in these attempts to effect a still further increase in the yield by the addition of free acid during growth as compared to its addition before inoculation are not conclusive. While experiments 28 and 29 would lead one to suspect that the optimum hydrogen-ion concentration for growth varies during the growth of the organism, experiments 30 and 31 do not seem to bear out this assumption. On the other hand, it must be admitted that the procedure followed in experiments 30 and 31 is not wholly comparable to that in experiments 28 and 29 with respect to the degree of the variations in acidity, imposed as they are in one instead of several days. I believe, therefore, that the results of experiments 28 and 29 should be given greater weight than those of experiments 30 and 31.

The contention of Nikitinsky (48) that *A. niger* eliminates a "stimulative" substance into the nutrient solution whose action is frequently inhibited by excessive increase in acidity of the cultures seems to me unproven. A glance at experiment 2 (of Nikitinsky) shows that the acidified cultures produce a greater yield (first harvest). Reference, moreover, to experiments 6, 7, 8, 9, and 11 indicates that in the majority of cases (see, however, *Penicillium griseum* grown on NH_4NO_3, experiments 7 and 11) when the acid formed in the first culture has been neutralized, the resulting yield in the second culture does not exceed that in the first. On the other hand, the yields for the second cultures, without neutralization of the acid formed by the first, show as a rule an acceleration of growth. The growth accelerations usually obtained through addition of excess $CaCO_3$, while without doubt in some cases due to a reduction in excessive acidity (experiment 23, culture 3) are in the majority of cases due to other causes, since previous observers (Wehmer, 68; Butkewitsch, 8) agree that a decrease in growth always occurs.

We may turn now to some results obtained by the use of a method of purification of the culture medium. The marked increase in yield following the addition of increased amounts of magnesium sulphate suggested the question whether the apparent action of the magnesium sulphate is due to

[The **Journal** for October (**8**: 311–356) was issued Nov. 20, 1919.]

the salt itself or to the impurities present in the salt. Recourse was had to further chemical purification.

The method of purification used depends on the fact that the "stimulants" in question are salts of cations whose hydroxides (and carbonates also) are quite insoluble and but weakly ionized. The barium carbonate method (66) of separation of ferric, aluminum, chromic, titanic, and uranyl salts from manganese, nickel, cobalt, zinc, and ferrous salts has long been known and is a standard method in the chemical laboratory. In using this method of separation it is necessary to carry out the hydrolysis in the cold, since on warming the salts of the bivalent metals are also hydrolyzed to an appreciable extent and are then precipitated by the barium carbonate.

It is evident, however, that the use of barium carbonate would be objectionable from a physiological point of view, since some barium necessarily goes into solution. Calcium carbonate, however, with use of higher temperatures (20 minutes at 14½ pounds in the autoclave) apparently causes the precipitation of these metals (iron, zinc, etc.) as effectually, and was therefore used in place of the barium carbonate.

The details of the method of treating the Pfeffer solution as I have used it are as follows: The Pfeffer solution is prepared by weighing out the sugar and salts (except iron sulphate) into the same beaker, redistilled water is added, and the compounds are permitted to go into solution. The solution is now placed in liter pyrex flasks, 15 grams of dry $CaCO_3$ (Baker's "Analyzed") are added per liter, and the neck of the flask is plugged with absorbent cotton. The contents are now thoroughly rotated so as to insure mixing and placed in the autoclave for twenty minutes at 14½ pounds pressure. On removal from the autoclave the flask is again rotated to cause mixing of the contents and set aside in a cupboard for 12 to 24 hours, or even for several days. When required for use it is necessary only to remove the cotton plug and to decant the supernatant liquid into a beaker. Fifty cc. of culture medium are now poured into each flask, the flask is plugged with absorbent cotton and sterilized. The substance to be tested, iron, zinc, etc., is added the next morning shortly before inoculation.

The Pfeffer solution so treated is still slightly acid to litmus, gives excellent tests for phosphates and sulphates, and without doubt contains calcium. The $CaCO_3$ residue contains appreciable amounts of phosphates and sulphates from the nutrient solution, but a test for sugar (Fehling solution) gave negative results. A slight precipitate forms in the treated solution on sterilization. The composition of this precipitate as well as that of the nutrient medium has as yet not been definitely determined, but it is planned, in connection with the testing of various other heavy metals and their combinations as substitutes for iron and zinc, to ascertain analytically just how and in what degree the components of the Pfeffer nutrient solution undergo modification as the result of the treatment. Reference to the earlier experiments given in this paper indicate that the variations in the proportions and concentrations of the Pfeffer nutrient solution which probably occur are not factors of primary significance.

With a culture medium so treated I have obtained on addition of both iron and zinc a dry weight of 1.158 g., and with the untreated Pfeffer solution a yield of 1.024 g. Both these values are maximum values for the W strain when supplied with iron and zinc. The maximum yield I have at any time obtained is 1.174 g. in culture with my original strain grown on Pfeffer solution and in Jena glass.

32. The effect of treatment of the magnesium sulphate as shown by its action on growth.

Strain W, 7			
(*A*) 10 : 5 : 130		(*B*) 10 : 5 : 130 ($MgSO_4 \cdot 7H_2O$ Purified).	
No Zinc	1.0 Mg. Zn/L	No Zinc	1.0 Mg. Zn/L
0.718 g.	0.757 g.	0.431 g.	0.785 g.
0.776	0.768	0.432	0.849
0.772	0.752	0.456	0.839
0.668	0.836	0.472	0.839
0.725	0.882	0.491	0.923
0.732	0.799	0.456	0.847

Solution (*B*) was prepared with $MgSO_4.7H_2O$ that had been autoclaved in 26% solution with 30 g. $CaCO_3$ per liter, the solution after treatment being decanted and used in making up the Pfeffer solution. The "no zinc" under solution (*B*) refers to the fact that none was added to the magnesium sulphate before its treatment; "+ 1.0 mg. Zn/L" refers to the fact that enough zinc was added to the magnesium sulphate before treatment so that in the preparation of the Pfeffer solution this concentration of zinc should result. That is, the 26 percent solution of $MgSO_4.7H_2O$ contained in the latter instance 2.0 mg. Zn/L before treatment with $CaCO_3$. The zinc cultures were sterile; excess zinc. p of ll cultures on harvest was 1–2.

The results of experiment 32 indicate clearly that the treatment with $CaCO_3$ acts essentially through a reduction of the zinc content of the $MgSO_4.7H_2O$. With solution (*A*), zinc is present in too high concentration; in solution (*B*), the treatment has apparently removed an appreciable amount of zinc, as is evidenced by the increased yield and increased fructification. As respects solutions (*A*) and (*B*) in those cases in which no zinc was added, we note that the treatment results in a reduction in yield. On the other hand, a comparison of the cultures in solution (*B*) with and without zinc would indicate that the action of the $CaCO_3$ cannot consist in any action on the $MgSO_4.7H_2O$, but must depend on the removal of the impurities present.

After decantation of the solution from the $CaCO_3$, the residue (15 g. $CaCO_3$ + 100 cc. Pfeffer solution) was used as a medium. An excellent "stimulated" culture resulted, a demonstration that the "stimulative" substances (zinc) had been removed from the Pfeffer solution by the $CaCO_3$. The cultures in the treated solution exhibited much scantier growth and spore formation than the control. The conidiophores in the former had apparently been reduced to a minimum length.

33. On the removal of added zinc from the culture medium by treatment with CaCO$_3$.

Strain W, 7			
	1.0 Mg. Zn/L + 30 g. CaCO$_3$/L		
Control	CaCO$_3$ Added Before Sterilization		CaCo$_3$ Added After Sterilization
	CaCO$_3$ Not Removed	Decanted Solution	
0.078 g.	0.545 g.	0.039 g.	0.759 g.
0.082	0.581	0.050	0.993
0.091	0.802	0.042	0.750
0.087	0.780	0.043	0.476
0.090	0.558	0.039	0.952
0.086	0.653	0.043	0.786

Cultures prepared from solution decanted from CaCO$_3$	
HNO$_3$	
0.1 cc./flask	0.084 g.
0.1	0.103
0.2	No growth
0.2	" "

As can be seen from experiment 33, the evidence for the removal of zinc by this method of treatment with CaCO$_3$ is quite conclusive. Furthermore, it is to be noted that the addition of CaCO$_3$ to a zinc culture (1 mg. Zn/L) depresses the yield least when added after sterilization, to a greater extent when added before sterilization, and most when it is added before sterilization and the supernatant liquid is removed by decantation. That is, the presence of calcium carbonate in the cultures causes a decrease in the rate of growth similar to that described by Wehmer (68), Butkewitsch (8), and Nikitinsky (48). If the calcium carbonate is added before sterilization, the effect of the heating in the sterilization process will be to cause the hydrolysis and precipitation of the zinc. The constituents of this residue go slowly into solution, thus affording zinc and iron necessary for the increased growth. If, however, the residue of calcium carbonate with its contained zinc, etc., is removed, as is the case when the solution is decanted, then growth is sharply limited. Indeed, in the latter case it is less than in the untreated Pfeffer solution.

I next tested whether the addition of nitric acid to the treated solution influences the yield. Two duplicates with 0.1 and 0.2 cc. HNO$_3$ per flask respectively were inoculated. In the latter no growth occurred, as was the case with the Pfeffer solution without treatment (experiments 20 and 23). With 0.1 cc. HNO$_3$ per flask, the growth was slightly accelerated, and, as was found to be the case with the untreated Pfeffer solution, the hyphae were opaque and the membranes wrinkled. Spore formation had been partially suppressed under these conditions. The small increase in growth on increasing the acidity indicates that the decreased acidity as a result of treatment cannot account entirely for the results observed.

With respect, therefore, to the marked acceleration of growth observed with very high concentrations of magnesium sulphate, I am of the opinion that the results observed are due to the presence of minute amounts of substances (probably zinc, iron, etc.) as impurities in the magnesium sulphate.

As I have already had occasion to state in a former publication (65) "it is perhaps questionable whether thus far anyone has grown *A. niger* in the complete absence of zinc." Accidental factors are exceedingly difficult to control, *e.g.*, variation in purity of a compound—even of different samples from the same bottle; contamination with dust, etc., from the air and from the cotton plugs of the flasks, etc.

The use of the hydrolytic purification method is well adapted for the study of the influence of zinc and iron on the growth of *A. niger*. Furthermore, by the use of this method I have been able to obtain a culture medium with which the non-addition of either iron or zinc markedly decreases the acceleration of growth by the other. This relation between iron and zinc is brought out in experiments 34 to 37 inclusive.

34. The effect of the addition of iron and zinc to the treated solution.
Each value for one culture.

Strain W, 11

	Treated Pfeffer Solution	
Composition of Solution	Yield	P_H
Control	0.018 g.	4
Control	0.013	4
+ 1 mg. $Fe_2(SO_4)_3$/flask	0.044	2–3
5 "	0.026	4
20 "	0.099	2–3
+ 1 mg. Zn/L	0.040	4
10 "	0.045	4
50 "	0.056	4
+ 1 mg. $Fe_2(SO_4)_3$/flask + 1 mg. Zn/L	0.731	1–2
1 " " " + 10 mg. Zn/L	0.787	1–2

The evidence shows that neither ferric sulphate even in 0.4 percent concentration nor zinc in a concentration of even 50 mg. Zn/L added *alone* can cause any but very slight increases in growth. If we add, however, but 0.002 percent $Fe_2(SO_4)_3$ and 1 mg. Zn/L *together*, we obtain the characteristic phenomenon of "stimulation." The high yields obtained by the combined action of zinc and iron have not been demonstrable by any other method of experimentation, neither has the markedly limited growth in the absence of zinc or of iron from the culture medium. I have further tested the possibility that the results with the treated solution are due to the calcium ion and the much lower acidity. The influence of the latter factor has been already emphasized. In experiment 35 I have attempted to duplicate the conditions with respect to acidity and the presence of calcium ion in the treated solution by the addition of ammonium hydroxide and $Ca(NO_3)_2$ to the untreated Pfeffer solution. The amount of $Ca(NO_3)_2$ is of course excessive in this solution as compared with the treated nutrient solution where the entire amount of $CaCO_3$ added amounted to only one and one half percent. The use of one percent $Ca(NO_3)_2$ was due to an error in mixing, it being estimated that a liter of solution had been prepared whereas in reality it was only a half liter.

35. The effect of increased acidity of the treated solution together with that of the addition of iron and zinc.

Strain W, 12

Yield	Control	Sporulation	H_3PO_4/Flask			Sporulation
	P_H		Yield		P_H	
0.012 g.	4	Fair	0.2 cc.	0.097 g.	2–3	Excellent
0.011	4	Fair	0.3	0.125	2–3	"
0.011	4	Fair	0.4	0.121	2–3	Fair
			0.5	0.041	2–3	Sterile

$Fe_2(SO_4)_3$/Flask

$Fe_2(SO_4)_3$	Yield	P_H	Sporulation
1 mg.	0.030 g.	3–4	Fair
5	0.033	3–4	"
20	0.326	2–3	Excellent
50	0.655	1–2	Sterile
100	0.823	1–2	"
200	0.864	1–2	"

Mg. Zn/L

Mg. Zn/L	Yield	P_H	Sporulation
1	0.115 g.	3–4	Fair
10	0.067	3–4	"
50	0.054	3–4	"
100	0.100	2–3	"
150	0.051	3–4	Practically sterile
200	0.055	3–4	" "

1 Mg. $Fe_2(SO_4)_3$/Flask

Mg. Zn/L	Yield	P_H	Sporulation
0.05	0.288 g.	2–3	Excellent
0.2	0.562	1–2	Good
0.5	0.752	1–2	Good
1.0	0.710	1–2	Sterile

0.1 Mg. Zn/L

$Fe_2(SO_4)_3$/Flask	Yield	P_H	Sporulation
0.5 mg.	0.473 g.	2–3	Excellent
1.0	0.727	1–2	Good
5.0	0.685	2–3	"

1 Mg. $Fe_2(SO_4)_3$/Flask

H_3PO_4/Flask	Yield	P_H	Sporulation
0.2 cc.	0.134 g.	2–3	Excellent
0.2	0.128	2–3	Excellent
0.4	0.110	2–3	Sterile

0.1 Mg. Zn/L

H_3PO_4/Flask	Yield	P_H	Sporulation
0.2 cc.	0.594 g.	1–2	Sterile
0.3	0.552	1–2	Sterile
0.4	0.337	1–2	Sterile

0.2 Cc. H_3PO_4/Flask

$Fe_2(SO_4)_3$/Flask	Yield	P_H	Sporulation
0.5 mg.	0.122 g.	2–3	Excellent
1.0	0.090	2–3	"
5.0	0.172	2–3	"
20.0	0.845	2–3	Sterile

0.2 Cc. H_3PO_4/Flask

Mg. Zn/L	Yield	P_H	Sporulation
0.075	0.654 g.	1–2	Sterile
0.2	0.477	1–2	"
0.5	0.456	1–2	"

Pfeffer Solution

Control

Mg. Zn/L	Yield	P_H	Sporulation
0	0.253 g.	3–4	Excellent
1	0.895	1–2	Good
10	0.909	1–2	"
25	0.972	1–2	"

+ 1% $Ca(NO_3)_2$ + 0.1 Cc. NH_4OH/Flask

Mg. Zn/L	Yield	P_H	Sporulation
0	0.398 g.	3–4	Excellent
1	0.850	1–2	Good
10	0.887	1–2	"
25	0.787	1–2	"

The $Ca(NO_3)_2$ used was the Baker's "Analysed." In both experiments 34 and 35 as well as in experiment 36 only 15 g. $CaCO_3$ per liter were used in effecting the purification.

Here again it is shown that the addition of both zinc and iron is necessary in order to obtain marked increases when the treated Pfeffer solution is used. Either alone in much higher concentration is apparently ineffective, growth being limited by the absence of the other. Nevertheless, with very high iron concentrations a marked acceleration of growth occurs. The same is also true for iron in acidified culture (0.2 cc. H_3PO_4 and 20 mg. $Fe_2(SO_4)_3$ per flask) as well as for zinc only. It should be emphasized that both the iron and the zinc salt used in these experiments are of a purity sufficient for analytical work but hardly come up to the required standard as respects physiological work of this character. Enough iron is still probably present in the treated solution together with that added with the zinc salt and in the spores to enable a limited (0.654 g.), but still marked, increase in growth on addition of zinc to take place. Asō (1) has reported that in the case of *Aspergillus Oryzae* the spores contain appreciable amounts of Fe_2O_3 (0.14 percent). With a similar iron content in the spores of *A. niger* it is quite evident that enough iron might be added to influence the results in view of the heavy inoculations necessary in these experiments for uniform results.[2] The increased acidity, since its action is to aid that of the zinc, makes its effect evident in the concentrations employed. This effect of increased acidity of the nutrient solution has already been noted in connection with the increased effectiveness of zinc in the untreated Pfeffer solution as well as in connection with the increased effectiveness both of iron and of zinc in the treated Pfeffer solution. This relation between acidity and the action of heavy metal appears to be of general significance, since, as already noted, it seems to hold also for the higher plants.

Inspection of the values obtained in the presence of calcium ion, in the Pfeffer nutrient solution of about the same acidity as the treated solution, shows that the influence of these two factors on the results obtained in the treated Pfeffer solution can be disregarded. Even in the presence of much more calcium than is present in the treated solution as well as of a decreased acidity corresponding approximately to the treated solution (slightly acid to litmus), the customary accelerations of growth with small amounts of zinc are obtained.

The next experiment (experiment 36) was planned to test the value to be placed on the accelerations of growth obtained in the presence of iron and zinc salt alone. In this experiment two Kahlbaum "Zur anal." compounds [$FePO_4$ and $Fe_3(PO_4)_2$] were used, the phosphates being selected for the very simple reason that neither the nitrates nor sulphates in a sufficient degree of purity were available. The relative insolubility of these salts is hardly a factor, since the addition of iron in soluble form results in the precipitation of the phosphates in the nutrient solutions.[3]

The spores were brown in many of the zinc-plus-iron cultures. Accompanying the brown spores, many papilla-like protuberances on the upper surfaces of the membranes were visible. These papillae have never been observed in zinc-free cultures, and in zinc cultures containing sufficient iron have been observed only at irregular intervals. Both the iron salts are the Kahlbaum "Zur. anal."; the zinc salt, the Baker's "Analysed."

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[2] Approximately 4 mg. dry weight of spores, etc., per flask.

[3] I have estimated that the amount of material (dry-weight) added at the time of inoculation is on the average approximately 3–4 mg. to each flask. The actual amount of course probably differed widely from flask to flask.

We note that the growth with either zinc or iron salts alone is sharply limited even with concentrations that may physiologically be termed very high. The presence of free acid in the cultures to which the iron salts are added, while it does cause accelerations of growth, can not in view of their limited magnitude be held as the factor responsible for the results with the treated solution. Again we note also that there can be no question that sufficient sugar is present in this solution to form a yield as great as that I obtained with the untreated Pfeffer solution (1.024 g.). The treated Pfeffer solution contains all the components that *A. niger* requires for growth with the exception of sufficient iron and zinc.

36. The effect of increased acidity of the treated solution together with that of the addition of iron and of zinc.

Strain W, 12. Treated Solution

$FePO_4$/Flask

$FePO_4$/Flask	Yield	P_H	Sporulation
0 mg.	0.004 g.	3–4	Fair
1	0.016	3–4	"
5	0.004	3–4	"
20	0.010	3–4	"
50	0.021	3–4	"
100	0.030	3–4	"
200	0.065	3–4	"

$Fe_3(PO_4)_2$/Flask

$Fe_3(PO_4)_2$/Flask	Yield	P_H	Sporulation
0 mg.	0.010 g.	3–4	Fair
1	0.032	3–4	"
5	0.045	3–4	"
20	0.094	2–3	Excellent
50	0.111	2–3	Good
100	0.147	3–4	"
200	0.213	3–4	"

0.2 Cc. H_3PO_4/Flask

$FePO_4$/Flask	Yield	P_H	Sporulation
0 mg.	0.117 g.	2–3	Excellent
1	0.123	2–3	"
5	0.157	2–3	"
20	0.154	2–3	"
50	0.187	2–3	"
100	0.277	2–3	"
200	0.273	1–2	"

$Fe_3(PO_4)_2$/Flask	Yield	P_H	Sporulation
0 mg.	0.071 g.	2–3	Excellent
1	0.106	2–3	"
5	0.176	2–3	"
20	0.170	2–3	"
50	0.254	2–3	"
100	0.437	2–3	"
200	0.580	2–3	"

0.1 Mg. Zn/L

$FePO_4$/Flask	Yield	P_H	Sporulation
0 mg.	0.076 g.	3–4	Fair
1	0.660	1–2	Excellent
5	0.822	1–2	Good
20	0.772	1–2	Good
50	0.840	1–2	Excellent
100	0.664	1–2	"
200	0.591	1–2	"

$Fe_3(PO_4)_2$/Flask	Yield	P_H	Sporulation
0 mg.	0.070 g.	3–4	Fair
1	0.553	2–3	Excellent
5	0.665	1–2	"
20	0.784	1–2	"
50	1.006	1–2	"
100	1.158	1–2	"
200	1.012	1–2	"

Mg. Zn/L

Mg. Zn/L	Yield	P_H	Sporulation
0	0.005 g.	3–4	Fair
0.025	0.047	3–4	"
1	0.079	3–4	"
5	0.037	3–4	"
10	0.030	3–4	Practically sterile
25	0.040	3–4	Practically sterile
50	0.037	3–4	Practically sterile
100	0.072	3–4	" "
150	0.040	3–4	" "
200	0.062	3–4	" "

My experiments with the treated Pfeffer nutrient solution indicate that iron and zinc are in a different class as to efficiency from any of the other "stimulative" elements (cobalt, nickel, etc.) so far tested. It seems probable from these experiments, further, that the large growths obtained apparently with the use of zinc alone in my earlier experiments (64, 65) were in reality due to the combined action of zinc and iron, the iron being introduced as an impurity with the sugar or the other salts together with that intentionally added (45). The very limited growth obtained in the treated solution when either is used alone indicates that only by their combined action could the very marked increase of growth obtained in the experiments referred to have been induced. A further study of the composition of the treated solution is necessary, but there can be no question that it still contains as noted the necessary salts and sugar. Experiment 35, and experiment 33 as well, show that neither the calcium ion nor any compounds which it may form has any particular effect on the growth. The evidence seems conclusive that the treatment really results in a more thorough removal from the medium of all salts of zinc and iron than had hitherto been achieved in such experiments on growth "stimulation." While the prolonged exposure to high temperatures (about 150° C.) of such a mixture as the Pfeffer solution with its sugar and salts and the $CaCO_3$ may lead to fundamental rearrangements, the chemistry of the process as used for the removal of iron and zinc is well understood and is utilized as a standard procedure for the separation of the trivalent from the bivalent metals of the ammonium sulphide group.

In the light of these experiments, I am therefore inclined to the opinion that the increased growth of *A. niger* which I have obtained in Pfeffer solution on addition of zinc salt, and which other workers (see review of the literature) have also obtained in this manner, is due not alone to the zinc added but to the fact that iron is present, in the same manner that the increased growth obtained in the presence of an iron salt is due not alone to the iron added but to the fact that zinc is present.

Lipman and Gericke (34) in a carefully made series of experiments find that when a sodium salt in toxic amount is added to the soil in pot experiments the injurious effects can be eliminated by the addition of copper and zinc salts. The thirteen tables of data submitted indicate that, as compared with the controls with alkali salt in toxic concentration, the presence of copper or zinc salt results as a rule in a greater dry weight of straw, grain, and roots. The importance of the practical application of these results as a means for the utilization of "alkali" soils is obvious.

The marked increases in yield obtained by these authors previously (33), under the same conditions except that no alkali salts were added, would indicate the possibility that in these as in their present investigations the acceleration in growth may be due to the action of copper, zinc, etc., primarily as "stimulants." That is, an acceleration in growth occurs in the presence of both non-toxic and toxic concentrations of "alkali" salts when zinc, copper, etc., are added. The effects of $CuCO_3$ in the presence of toxic concentrations of Na_2CO_3 is, as compared to that obtained with $NaCl$ or Na_2SO_4, not marked according to the authors. In cultures of *Aspergillus niger* the effect of the addition of substances causing a decrease in acidity is, as shown in experiments 23, 25, 26, etc., markedly to decrease the "stimulative" action of zinc. Zinc salts and acidity have a mutual effect on growth, reproduction, etc., in cultures of *A. niger*. Acidity within certain limits markedly favors the growth of *A. niger*. Acidity of the soil is supposed to be detrimental to cereal crops, but here again probably only when it exceeds certain limits. If, as I believe to be the case, the facts determined for the nutrient solution with *Aspergillus niger* hold also in general for the higher plants, then maximum growth will be best assured by a relatively (compared to the optimum of the particular plant) lower acidity and the presence of heavy metals (iron, zinc, manganese, etc.) in predetermined amount. The practical application of these facts to diminish the action of heavy metal salts in toxic concentrations is well shown, even if unconsciously, by the practise of applying a good dressing of lime as an effective antidote (60, p. 51) whenever under special conditions infertility is traced to any of these metallic salts. The decreased acidity, perhaps also the partial precipitation of the heavy metals present in the soil solution, leads to increased growth both by decreasing their concentrations and by a marked diminution of the action of the heavy metals resulting from the increased alkalinity.

My observations on the acceleration of growth by increased acidity, etc., are of interest in connection with MacDougal and Spoehr's (36) studies on the correlation between growth and the imbibition of water by colloids as influenced by variation in the acidity. Growth in Opuntia, they conclude, is decreased by the excessive accumulation of acids in darkness. The decreased acidity of the tissues due to the action of light during the forenoon is accompanied by an acceleration of growth. Furthermore, the imbibition by protein and carbohydrate colloids, and by mixtures of the two in varying proportions, differs as to acid, water, and alkali. The imbibition phenomena of gelatine, they find, parallel more closely the growth phenomena of the animal organism; whereas those of agar parallel more nearly the growth phenomena of plants. One of their colloidal mixtures compounded—gelatine 100 + agar 1—reacts as regards imbibition in the presence of acid, of water, and of alkali in a manner greatly similar to the growth rates of *A. niger* under similar conditions of variation in the reaction of the medium. Thin plates of this mixture, they find, increase in thickness to 520 percent of the original thickness in *n*/100 NaOH, to 750 percent in water, and to 1100 percent in *n*/100 HCl.

It is always to be remembered, however, that in *A. niger* we have increased assimilation of food materials as shown by the increased dry weight.

The phenomenon of imbibition of water by colloids has also been used by Fischer (15) in the interpretation of oedematous swellings in the animal organism. The imbibition of water by colloids like gelatine, gluten, etc., in the presence of various compounds and degrees of acidity is paralleled, he shows, to a high degree by the behavior of organized animal tissues under the same conditions. Whereas, however, the use by Fischer of certain acidities led presumably only to an increased water content, so that the total dry weight is not increased, in *A. niger*, as just noted, under analogous conditions an increase in the dry weight results. That perhaps increase in dry weight occurs to some extent through hydration of the colloids, as has been found by Pauli to take place with gelatine, is not, however, improbable. Still, while there may be some question whether mere hydration can be referred to as growth, there can be no doubt that we have acceleration of true growth in *A. niger*.

The growth accelerations of *A. niger* resulting from "chemical stimulation" are hardly to be attributed in their entirety to, nor apparently are they paralleled by, the imbibitional capacity of the fungus for water. Growth as we are studying it involves *cell multiplication* and *increase in weight* of organic compounds. The root-tip has long served as the classical object for the illustration of the different kinds of growth: cell division, increase in dry weight, and increase in volume; not to mention differentiation. At the apex the cell divides, increases in volume here being due to increases in the organic compounds of the cell, and hence in dry weight due to the imbibition and assimilation of foods and water. Farther back, in what is commonly referred to as the zone of elongation, the rapid increase in size of the cells appears in the main to be due to absorption of water and its accumulation in vacuoles, without necessarily an increase in the dry weight of protoplasm present. Lastly we note that certain cells undergo a still further modification of form in the zone of differentiation. The distinction between the different kinds of growth is not always sharply marked. Neither are the primary forces making for the intake of water and foods by the cell determined as yet with any certainty. An increase in protoplasmic volume may be attributed to an increased imbibition of water, just as an increase in size of the vacuoles is attributed to osmotic phenomena. The absorption of foods by the organism results in the formation of colloids, which, however, would not necessarily increase the imbibitional capacity of the protoplasm. Those materials which accumulate as soluble bodies in the vacuoles and lead to increase in size of course play a very important part in the elongation of the so-called growing region of the root. In *A. niger* the thickening of the cell walls (differentiation) also probably contributes largely to the end results of the growth acceleration in the presence of zinc and iron. It is certainly of interest that in animal tissues, in tissues of cacti, and in the hyphae of *A. niger*, increase in acidity within certain limits (*i.e.*, optimum p_H or acidity) may lead to increased absorption of water, or of nutrients that are further elaborated, or of both.

It is clear that increased acidity and the specific effect of certain heavy metals are not the sole means by which growth is accelerated and spore formation retarded or suppressed in the case of "chemical stimulation" of *A. niger*. Such compounds as chloroform, ether, etc., which act as "stimulants," do not, as far as we at present know, increase the acidity of the nutrient solution. No purpose is served by speculations attempting to suggest uniformity where none may exist.

My studies were made under the direction of Professors W. G. Marquette and R. A. Harper. To both I am greatly indebted for advice, aid, and encouragement.

Summary

1. Increased acidity of the Pfeffer nutrient solution within a certain range results in the exhibition in *Aspergillus niger* cultures of growth "stimulation" like, but less in amount than, that obtained by addition of salts of certain heavy metals. The appearance of the cultures duplicates exactly, in decrease in sporulation and in formation of opaque hyphae resulting in thick, white, wrinkled membranes, that of the classic zinc cultures.

2. Increase in acidity of the nutrient solution supplements the action of heavy metals (zinc, iron); decrease in acidity detracts from the action of heavy metals.

3. Salts of the heavy metals which act as "stimulants" hydrolyze as a rule in aqueous solution so as to result in an increased acidity, and this increase in acidity, though slight, owing to the low concentration in which the salts are used, may possibly be considered as rendered effective through membrane concentration. Sodium silicate is an exception to this rule of increase in acidity by "stimulants," and it was found in my experiments not to produce effects like those of zinc on the growth, sporulation, etc., of *A. niger.*

4. The progressive increase in acidity of the culture consequent upon the addition of a "stimulant" is, at least in major part, a result of the activities of the organism, and may in turn be concerned also in the acceleration of growth and in the retardation of spore formation.

5. Autoclaving at 14½ pounds the Pfeffer nutrient solution with calcium carbonate leads apparently to the removal to a high degree of the remaining traces of iron and zinc, and probably of all heavy metals present.

6. Evidence is given that in the Pfeffer nutrient solution thus treated, practically no growth takes place; that the addition of either iron or zinc alone causes only a very slight increase in growth, but that when zinc and iron are added together a marked increase in growth takes place.

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AN INDEX OF HARDINESS IN PEACH BUDS

Earl S. Johnston

The effect of various environmental conditions on hardiness of the peach is an important question to the fruit grower, but a thorough study of this subject can not be made until some criterion is found for determining the degree of hardiness. A most desirable criterion would be some physical or chemical measurement in which personal judgment is reduced to a minimum. After such a measuring unit has been found it will then be possible to determine more accurately the effect of environmental conditions on hardiness and the degree of hardiness in different varieties growing under the same conditions.

Ohlweiler,[1] Chandler,[2] and a number of other workers have found a correlation between the density of cell sap and the resistance of the plant to low temperatures. In measuring osmotic pressures and freezing point lowerings of expressed cell saps a number of important conditions are necessarily neglected. The size and shape of cells and the properties of the colloids present undoubtedly exert a most important influence. On the former depend the size and shape of capillary water films and on the latter the amount of water held by imbibition. Both limit the degree of hardiness of the tissue. These facts may in part explain the lack of correlation observed by other workers such as Salmon and Fleming,[3] who state that:

> "There appears to be no relation between the cryoscopic value of the extracted sap of winter rye, wheat, emmer, barley, and oats grown in the field with normal conditions and their ability to resist winter killing. Turgidity of the tissue as influenced by physiological drought appears to have more influence than the kind of grain on the concentration of the cell sap. Harris and his co-workers[4] have pointed out that the environmental conditions under which the plant grows seem to modify the osmotic pressure of the cell sap."

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[1] Ohlweiler, W. W. The relation between the density of cell sap and the freezing points of leaves. Rep. Mo. Bot. Gard. **23**: 101–131. 1912.

[2] Chandler, W. H. The killing of plant tissue by low temperature. Mo. Agr. Exp. Sta. Res. Bull. 8. 1913.

[3] Salmon, S. C., and Fleming, F. L. Relation of the density of cell sap to winter hardiness in small grains. Journ. Agr. Res. **13**: 497–506. 1918.

[4] Harris, J. A. On the osmotic concentration of the tissue fluids of phanerogamic epiphytes. Amer Journ. Bot. **5**: 490–506. 1918. Harris, J. A., and Lawrence, J. V., with the coöperation of R. A. Gortner. The cryoscopic constants of expressed vegetable saps as related to local environmental conditions in the Arizona deserts. Physiol. Res. **2**: 1–49. 1916.

Beach and Allen[5] state that maturity bears an important relation to hardiness and that the amount of moisture present in tissues is an important factor relating to maturity. These writers found that the moisture content is slightly lower in twigs of the more hardy apple varieties than in tender ones. Shutt[6] gives a series of determinations of moisture content of apple twigs which compares very favorably with a series arranged according to hardiness, the more tender containing the greater percentage of water. Thayer[7] notes the general rule that buds on young trees are more apt to be injured than those on more matured trees. This is no doubt closely related to the water content of the tissues. Daily variation in water content of leaves and twigs has been observed in several cases,[8] and in all probability a change in water content of plant tissues and organs is a most important factor influencing the response of the plant to its environment.

An experiment was carried out in the winter of 1918–1919 with the object of determining the moisture content of fruit buds taken from two varieties of peach, one of which is commonly considered relatively more hardy than the other. The two varieties selected for this study were the Elberta and Greensboro. Fifteen trees of each variety chosen for this work formed part of a nine-acre peach orchard. Four trees of each variety were situated on high ground, three on low ground, and seven trees were growing under various fertilizer treatments. Beginning with November 8, 1918, and thereafter at monthly intervals, except for the last period which was three weeks in length, buds were collected from these trees. The samples were taken from the same marked branches of the selected trees on clear days that were preceded by at least one clear day. This precaution was taken in order to avoid getting the buds when they were in an excessive moist condition following a rain or fog. Likewise, the afternoon was selected as the time of day for collecting the samples. The samples were immediately placed in small medicine vials with screw caps and weighed the following day in the laboratory. Each sample consisted of ten fruit buds. After determining the green weights, the buds were placed in a vacuum electric oven and dried to constant weight at a temperature of approximately 82° C. and a partial vacuum of 40 to 65 cm. of mercury. The drying usually required from 14 to 18 hours. The dry weights were then determined.

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[5] Beach, S. A., and Allen, F. W., Jr. Hardiness in the apple as correlated with structure and composition. Iowa Agr. Exp. Sta. Res. Bull. 21. 1915.

[6] Shutt, F. T. On the relation of moisture-content to hardiness in apple twigs. Proc. & Trans. Roy. Soc. Canada, II. **9**: sec. 4: 149–153. 1903.

[7] Thayer, P. Winter killing of peach buds. Ohio Agr. Exp.Sta. Mo. Bull. **1**: 311–312. 1916.

[8] Livingston, B. E., and Brown, W. H. Relation of the daily march of transpiration to variations in water content of foliage leaves. Bot. Gaz. **53**: 309–330. 1912. Lloyd, F. E. The relation of transpiration and stomatal movements to the water content of leaves in *Fouquieria splendens*. Pl. World **15**: 1–14. 1912. Shreve, Edith B. The daily march of transpiration in a desert perennial. Carnegie Inst. Wash. Publ. 194. 1914.

Data showing the seasonal variation in the average green weight, dry weight, ratio of water content to green weight, and ratio of water content to dry weight of buds taken from the fifteen trees of the two varieties are presented in table 1. The arrangement is such that corresponding values of the two varieties may be easily compared as well as the values for different periods. The average green and dry weights represent the weights of ten buds. With the exception of the average dry weights of buds collected on November 8, all values representing the Elberta variety are greater than those representing the Greensboro. These higher values are mostly due to the greater amount of water contained in the buds. (It is assumed that in the drying process the amount of water lost makes up the largest part of the substances driven off and that no oxidation processes have taken place to change appreciably the dry weight values.) Both the green and dry weights of each variety increase month by month, slowly during the winter, but very rapidly in early spring.

TABLE 1. *Data showing seasonal variation in average green weight, average dry weight, average ratio of moisture content to green weight, and average ratio of moisture content to dry weight of fruit buds of the Elberta and the Greensboro peach.*

Date Samples Were Collected	Ave. Green Weight		Ave. Dry Weight		Ratio of Water Content to Green Weight		Ratio of Water Content to Dry Weight	
	Elberta	Gr'boro	Elberta	Gr'boro	Elberta	Gr'boro	Elberta	Gr'boro
	Grams	*Grams*	*Grams*	*Grams*				
November 8.......	.124	.121	.073	.073	.41	.40	.69	.65
December 6.......	.144	.129	.079	.073	.46	.43	.84	.76
January 7.......	.144	.123	.082	.075	.43	.38	.76	.62
February 7.......	.164	.128	.082	.075	.49	.42	.99	.71
March 7.......	.327	.220	.115	.092	.65	.58	1.85	1.37
March 28.......	1.050	.750	.205	.180	.80	.76	4.12	3.17

The differences between the values of the two varieties as well as the seasonal variations of each are perhaps more clearly seen in figures 1 and 2, representing these same data plotted as ordinates. Time periods are shown as abscissas. The seasonal variation in dry weight is small in comparison with that of green weight. Increase in green weight is thus seen to be apparently due to the water. The ratio graphs (fig. 2) based on dry weight show greater seasonal variations and greater differences between the two varieties than those based on green weight.

Ratios of water content to dry weight of fruit buds from individual trees are given in table 2 in order to facilitate comparison between trees of the two varieties that were similarly situated. The first seven trees had received fertilizer treatment about May 1 of each of the three preceding years. The fertilizers used were acid phosphate (16 percent), muriate of potash, and nitrate of soda, represented by *P*, *K*, and *N* respectively. Whether used singly or in combination, the same amount of each fertilizer was applied per tree receiving such treatments. These amounts were:

2 pounds of acid phosphate, 1 pound of muriate of potash, and 1½ pounds of nitrate of soda. The check trees receiving no fertilizers are designated by the letters *C* and *CH*. Trees growing on high ground are represented by the letter *H* and those on low ground by *L*. The trees treated with fertilizers were on comparatively high ground, and one of the check trees

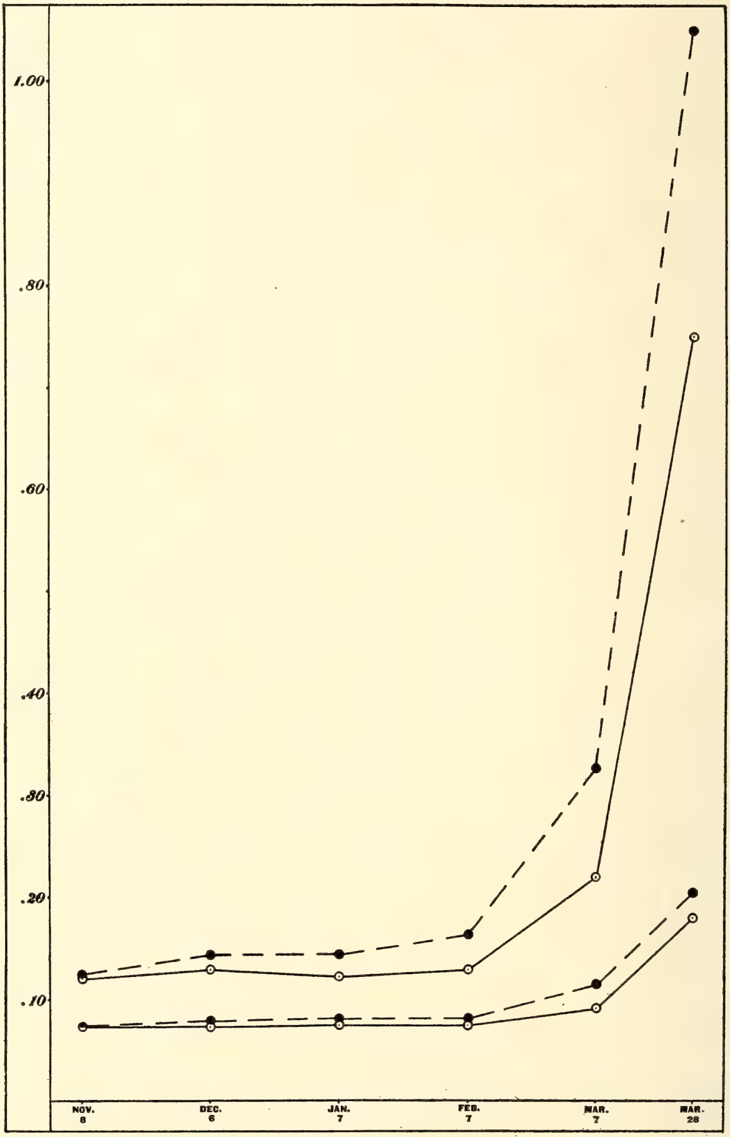

FIG. 1. Graphs showing seasonal variation in green weight (upper pair) and dry weight (lower pair) of Elberta (broken lines) and Greensboro (continuous lines) fruit buds.

(*CH*) is included in the group growing on high ground. In all but two cases, trees of the two varieties designated by the same letter were growing 18 feet apart. In the two exceptions the trees were within 38 feet of each other.

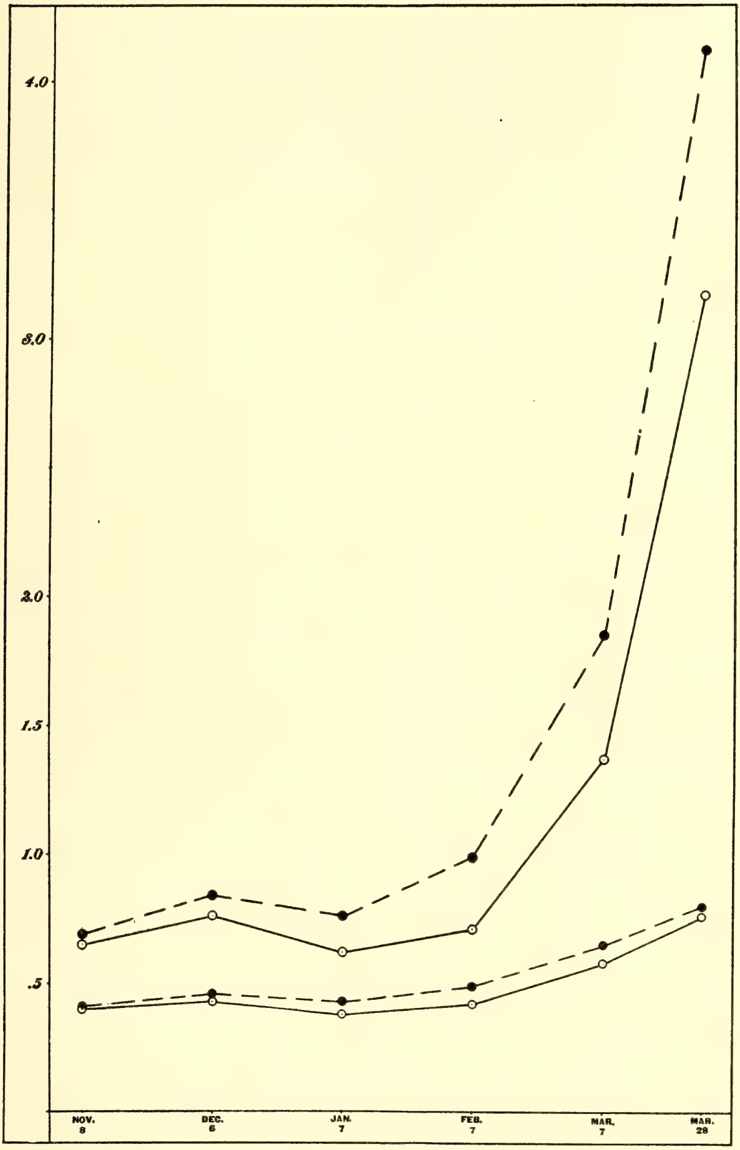

FIG. 2. Graphs showing seasonal variation in ratio of water content to dry weight (upper pair) and ratio of water content to green weight (lower pair) of fruit buds in the Elberta (broken lines) and Greensboro (continuous lines) peach.

TABLE 2. *Data showing seasonal variations in ratio of water content to dry weight of fruit buds from individual trees of the varieties Elberta and Greensboro.*

Tree	November 8		December 6		January 7		Feburary 7		March 7		March 28	
	E	G	E	G	E	G	E	G	E	G	E	G
P.	.68	.63	.82	.79	.74	.63	.98	.71	1.80	1.35	4.21	3.30
K.	.67	.66	.82	.73	.76	.60	1.00	.68	1.86	1.24	4.23	2.91
N.	.65	.58	.82	.73	.72	.56	.89	.71	1.68	1.36	3.76	3.45
PK.	.70	.67	.85	.73	.81	.62	1.10	.69	1.97	1.24	4.28	2.75
PN.	.70	.64	.88	.74	.83	.60	1.07	.70	1.97	1.44	4.21	3.11
KN.	.75	.69	.89	.78	.82	.66	1.20	.72	2.01	1.32	4.18	3.22
PKN.	.81	.66	.90	.78	.86	.67	1.11	.83	1.95	1.39	4.07	3.00
Ave. .	.71	.65	.85	.75	.79	.62	1.05	.72	1.89	1.33	4.13	3.11
C.	.74	.66	.89	.75	.76	.54	.97	.70	1.97	1.29	4.19	3.61
CH.	.74	.61	.85	.74	.72	.60	.99	.70	1.94	1.34	4.19	2.95
Ave. .	.74	.64	.87	.75	.74	.57	.98	.70	1.96	1.32	4.19	3.28
CH.	.74	.61	.85	.74	.72	.60	.99	.70	1.94	1.34	4.19	2.95
H.	.64	.72	.81	.81	.64	.64	.85	.71	1.88	1.45	3.79	3.45
H.	.67	.70	.81	.76	.80	.67	1.02	.71	1.91	1.40	4.25	3.10
H.	.66	.65	.78	.75	.78	.65	.96	.66	1.68	1.39	4.06	3.04
Ave. .	.68	.67	.81	.77	.74	.64	.96	.70	1.85	1.40	4.07	3.14
L.	.63	.63	.82	.73	.64	.57	.78	.68	1.54	1.27	4.11	3.01
L.	.63	.64	.80	.81	.69	.67	.83	.77	1.71	1.75	4.05	3.65
L.	.72	.68	.89	.73	.77	.66	1.06	.74	1.89	1.34	4.20	3.07
Ave..	.66	.65	.84	.76	.70	.63	.89	.73	1.71	1.45	4.12	3.24

It is of interest to note that the amount of moisture in proportion to the dry weight of the Elberta fruit buds is greater than that of the Greensboro buds in almost every case. There are, however, a few exceptions. On November 8, the Greensboro values were greater for two of the trees on high ground and for one tree on low ground. The Greensboro values are also greater on December 6 and again on March 7 for this same tree growing on low ground. There are three cases in which the values of the Greensboro equal those of the Elberta: one on November 8 for a tree on low ground, one on December 6 for a tree on high ground, and one on January 7 for the same tree on high ground. There is no uniformity in the differences between trees receiving fertilizer treatments. Some differences are seen, but these are probably due to individual variations. No great differences are apparent between the ratio values of buds taken from trees growing on high ground and from those growing on low ground.

CONCLUSIONS

Attention is called to two points in these observations. First, there is a marked seasonal increase in the water content of fruit buds of the Elberta and Greensboro peach whether determinations from individual trees or from averages are considered. Second, as the season advances the difference between the water content of fruit buds of the Elberta and Greensboro peach becomes more marked, the values for the Elberta being the greater. Further investigation will be necessary to determine whether or not these differences are characteristic of the varieties, and to what extent such moisture content values are related to hardiness of these fruit buds. Attention is also called to the fact that of these varieties the Greensboro is considered more hardy with respect to winter injury, and to the fact that in this same variety the ratio of water content to dry weight of fruit buds is considerably less than that of the Elberta. Early spring is a critical time in the peach industry because of the "tender" condition of fruit buds. This same period is the time when the moisture content of the buds increases very rapidly.

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NOTES ON THE DASHEEN AND CHAYOTE

HEBER W. YOUNGKEN

Within comparatively recent years, the United States Department of Agriculture has introduced into southern horticulture two exotic vegetables, the Trinidad dasheen and the chayote. The success attending their experimental culture, and the steadily increasing demand by the populace of many sections, have encouraged their commercial cultivation to a limited degree. It may be safe to predict, however, that when the delicacy of their flavor becomes more generally known, they will be cultivated to such an extent as to be common articles in our markets alongside the potato and the squash.

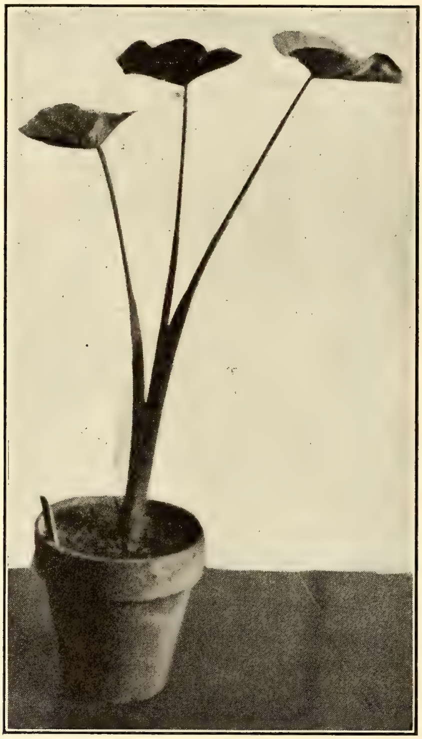

FIG. 1. Three-months-old plant of the Trinidad dasheen, *Colocasia esculenta* (L.) Schott, as grown in the greenhouse of the Philadelphia College of Pharmacy.

THE TRINIDAD DASHEEN

The Trinidad dasheen was introduced into the United States from the Island of Trinidad, West Indies. Its native home was probably China, partly because certain varieties of the taro closely allied to it have been found growing in that country and partly because its name appears to be a corruption of the French phrase "*de la Chine*."[1]

The plant is a variety of *Colocasia esculenta* (L.) Schott, a member of the Araceae, and closely related to the common elephant's ear plant of our gardens. Its underground parts (fig. 3) consist of a large central corm weighing from two to four pounds, of spheroidal or broadly fusiform shape and reddish brown color, and, in addition, numerous lateral cormels, which spring from various nodes along the periphery of the mother or central corm. Both mother corm and lateral cormels are marked by the presence of numerous rings which represent leaf scars. When the lateral cormels are removed, large circular to ovate, light-colored spots are exhibited. The total from one hill of these underground portions ranges from 4 to 30 pounds. The aboveground parts (figs. 1, 2), consist of several petiolate, auriculate, peltate, bright green leaves, three feet or more long, and a spadix, which is free and terminated by a sterile appendage.

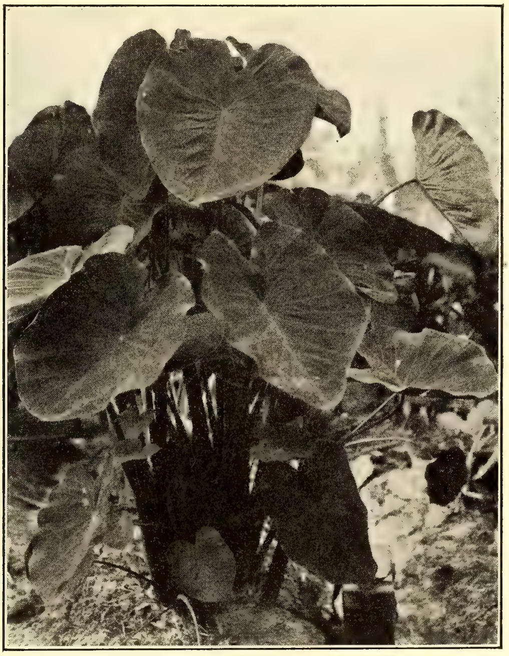

FIG. 2. Mature plant of the Trinidad dasheen, as grown under field culture in Florida.

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[1] Young, R. A. The dasheen; its uses and culture. Sep. 689, Yearbook U. S. Dept. Agr., 1916.

Histology

When examined microscopically, sections of the Trinidad dasheen corm, passing from the periphery toward the center, show the following histological peculiarities:

1. A zone of cork composed of numerous layers of cells with suberized walls, varying in size from irregular polygonal to rectangular.
2. A broad zone of phellogen, composed of more or less rectangular, tangentially elongated cells with rich protoplasmic contents.
3. A broad central matrix composed of parenchyma, the cells of which are mostly thin-walled and abundantly filled with starch. The starch grains are mostly simple, but compound grains composed of as many as eight units are occasionally met with. The simple grains vary in outline from rounded to irregularly rounded to irregularly ovate or angular. Some of these are devoid of striations or distinct hilum, while others show both of these structures. In size, they range from 3 μ to 19.2 μ. The hilum, when distinct, varies from linear to circular to angular to several-cleft. The lamellae and striations, when distinct, are always concentric. These, as well as the hilum, may be well observed in a mount stained with dilute gentian violet. Scattered throughout this region are to be noted numerous mucilage reservoirs of irregularly rounded, oval or ellipsoidal outline, whose contents are deeply stained with basic aniline dyes. The fibrovascular bundles are of concentric type and may be found scattered throughout the section in irregular fashion. From the main axis bundles, numerous branch bundles emanate at various levels, which course out into the lateral cormels. Crystals of calcium oxalate are found in numerous cells of the central matrix in the form of raphides.

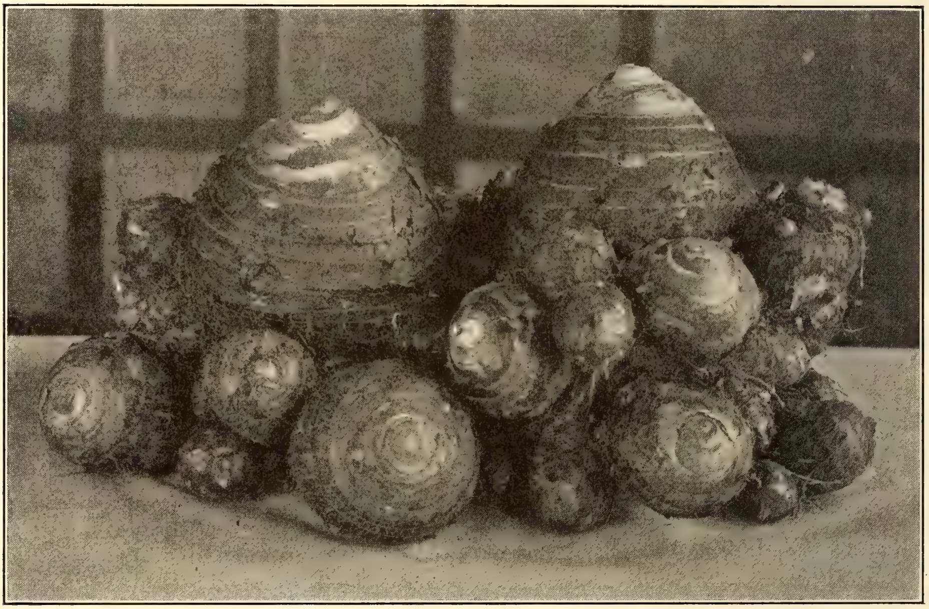

FIG. 3. Two mother corms with their lateral cormels, the product of an eleven-pound hill of Trinidad dasheens (photo. by R. A. Young).

Uses of the Dasheen

The portions of the plant suitable for diet are the corms with their lateral cormels and the aerial shoots. The former are not intended to replace the white or the sweet potato, nor the latter the asparagus, but rather to augment the comparatively small number of starchy vegetables now in use in our country. The underground parts, which are sold as "dasheens" in some of our markets, contain about 50 percent more protein and 50 percent more starch and sugars than the potato tuber. The average of ten analyses of these portions made by the Department of Agriculture is as follows:

	Percent
Solids	37.235
Ash	1.3
Starch	26.097
Soluble sugar	1.75
Ether extract	.157
Crude fiber	.71
Proteids	3.03
Pentosans	1.24

The corms and cormels are employed in the same manner and in quite as many ways as the white potato. When baked or boiled, the interior of a mature specimen is mealy, though firmer than the potato, because of its comparatively lower water content. Its flesh varies in color from cream to more frequently grayish-white or tinged with violet. Dasheens are best eaten directly after they have been baked or boiled. If kept standing, they gradually lose in palatability.

An excellent flour has been made from dasheens. The corms and larger cormels are pared and either sliced or shredded and then dried and ground in a mill. This flour is mixed with that of wheat or rye in the proportion of one part of the former to three or four parts of the latter.

The shoots are said to be more tender than those of asparagus. These are blanched, before being used, by forcing them from larger corms in the dark.

The Chayote

This vegetable, concerning which little has been recorded, is the fruit *Chayota edulis* Jacq., a native of tropical America. The plant (fig. 4) is a climbing, sparsely hairy vine, with perennial tuberous roots. Its stem bears alternate, cordate, palmately three-lobed or -angled leaves, which are membranous in texture. From points along the stem opposite the leaves 2–5-branched tendrils arise, which assist the vine in climbing. The flowers are monoecious and axillary; the pistillate are solitary, while the staminate are borne in small clusters. The calyx tube is crateriform with a five-lobed limb. The greenish to cream-colored corolla is rotate, deeply five-parted, the segments being ovate-lanceolate. The filaments and styles are connate into a central column of which 2-celled anthers appear as lobes. The stigmas are closely set together, forming a small head. The ovary is inferior. The fruit is a greenish or ivory-white, fleshy, pear-shaped, or globose, one-seeded pepo. Its surface is more or less corrugated and marked by the presence of spines around both ends. The embryo protrudes from the center of the distal end (fig. 5) before the fruit is mature. The seed is exalbuminous and consists of a seed coat firmly adherent to the endocarp and enclosing two cotyledons, a plumule, and a radicle. The cotyledons attain a length of from 2 to 2½ inches, which is on the average one-half the length of the fruit. The average weight of the fruit is about eight ounces.

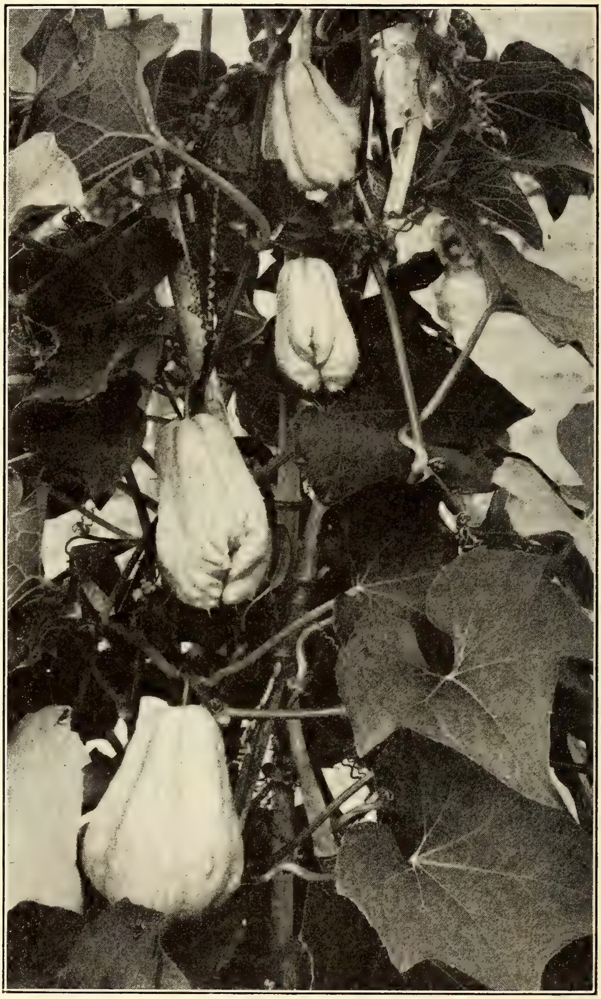

FIG. 4. Plant of the chayote, *Chayota edulis* Jacq., as grown under field culture in the South by the Bureau of Plant Industry, U. S. Department of Agriculture.

According to a circular issued by the United States Department of Agriculture, the chayote may be grown successfully on any well drained, cultivated lands in those sections of the southern states where the ground does not freeze—anywhere south of a line drawn from Charleston, South Carolina, to Baton Rouge, Louisiana; and along the Gulf coast of Texas. It has fruited at some points north of this.[2] It is reported to have been grown in California.

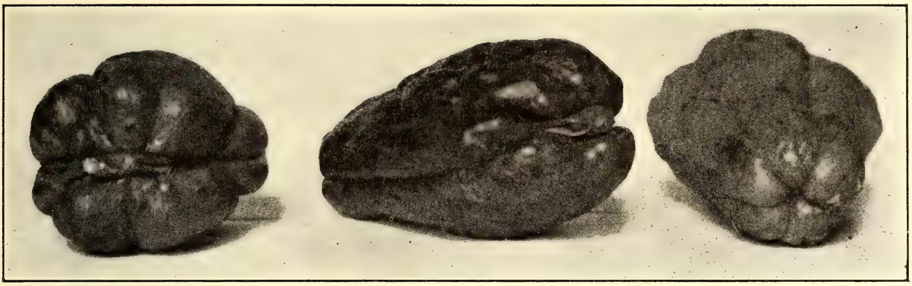

FIG. 5. Fruits of the chayote, one-third natural size. Note, from left to right: distal, lateral, and proximal aspects. The embryo is seen protruding from the distal ends of two of the fruits.

Histology of the Fruit

Alike with other cucurbitaceous fruits, that of the chayote agrees in the fusion of the receptacle with the carpellary portions during the developmental process.

The receptacle constitutes by far the greater portion of the fruit area. In surface section, the outer epidermal cells are polygonal in outline and richly protoplasmic. Many contain small prisms of calcium oxalate. Scattered all over this region and interspersed among the regular epidermal cells may be noted small groups of cells, not unlike the other cells in shape, but having thicker walls and yellowish to light brown fixed oil contents. Stomata may also be found in moderate numbers in this region. These with their guard cells are broadly elliptical in outline. Each is surrounded by five neighboring cells. In cross section the outer walls of the epidermal cells are slightly convex and cutinized. Beneath the outer epidermis is a zone of several layers of parenchymatous cells, many of which have lignified walls. In some instances, lignification occurs in the walls of the cells directly underneath the epidermis; in others the lignified elements are separated from the epidermis by one to several layers of cells with non-lignified walls.

The next broadest zone of the receptacle is composed of more or less radially elongated, thin-walled parenchyma cells, comparatively small in the outer region but gradually becoming larger toward the center. Numerous branched latex tubes with yellowish contents course irregularly through this region. Fibrovascular bundles of the bicollateral type are also to be noted. The most conspicuous elements of these regions are the spiral ducts which attain a breadth of 28.6 microns.

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[2] Circular on chayote. U. S. Dept. of Agric.

Separating the receptacle from the carpellary portion of the fruit may be noticed a sharply delimited band of cells, three layers thick. Of these the outer layer and inner layer are comparatively clear; the middle layer is filled with dense protoplasmic contents. The innermost layer of cells of this region is the broadest, contains starch grains, and doubtless represents the epicarp of the ripened carpellary wall.

Passing from this region toward the embryo, numerous layers of thin-walled cells are noted, of rounded or irregular outline, whose lumina contain either protein or carbohydrate contents or both. This region constitutes the mesocarp. It is traversed by numerous bicollateral bundles. The endocarp consists of a layer of rather small tangentially elongated cells. Over that portion of this region which is unattached to the seed coat, the cells are larger and have very thick brownish walls.

Seed

The seed coat is composed of tangentially elongated cells, the outer walls of which are united firmly to a portion of the endocarp.

Cotyledons

The outer covering tissue or epidermis consists of a layer of cells which in surface view are polygonal, and rectangular when observed in transverse section. Many of the cells of this tissue possess starch grains. Branched stellate hairs and glandular hairs are scattered over this tissue.

Beneath the epidermis is a spongy parenchyma composed of somewhat spheroidal to polygonal cells containing starch grains, which are mostly simple, spheroidal or plano-convex, rarely 2–3-compound. These have an ordinary range of 3 to 28 microns in diameter. Occasionally, somewhat elongated ovoid grains are seen which attain a length of 40 microns.

Radicle

This shows the usual structures typical of this portion of the cucurbitaceous embryo. The cells of the cortex are rich in protoplasm, have prominent nuclei, but are entirely devoid of starch.

Uses of the Chayote

The fruits should be picked from vines when but two thirds or three fourths grown. They lose their delicate flavor and become tough if allowed to mature. They are then employed similarly to the squash. The vines, tuberous roots, and fruits may be used as fodder for stock. The woody stems furnish a fine fiber known to the French as "*paille de chouchon.*"

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THE DEVELOPMENT OF THE GAMETOPHYTE AND THE DISTRIBUTION OF SEXUAL CHARACTERS IN *FUNARIA HYGROMETRICA* (L.) SCHREB.

Mabel Mary Brown

Introduction

The conflicting statements published concerning the sexual conditions in *Funaria hygrometrica* suggested the problem of determining by experimental methods the range of possibilities in the distribution of sex organs in this species.

The reproductive organs of the Bryophytes were first clearly recognized as such by Hedwig (1782). He designated the sexual conditions found in the mosses, by analogy with those of the higher plants, as *hermaphroditic*, *monoecious*, and *dioecious*. This classification has been retained until the present time with the addition of several new terms. Schimper (1860, p. 13) added the term *polygamy*, applicable to that condition in which the male and female organs of a moss may be borne on the same plant or on different ones. Lindberg (1882) characterized those hermaphroditic species in which the archegonia are scattered among the antheridia and the entire group is enclosed by bracts as *synoicous;* he gave the designation *paroicous* to those in which the archegonia are isolated at the apex of an axis and the antheridia are borne in the axils of the leaves. He applied the term *autoicous* to monoecious mosses in which the sex organs are borne on separate branches of the same plant. He recognized four conditions of autoicism known as *cladautoicism*, *rhizoautoicism*, *gonoautoicism*, and *pseudoautoicism*. Combinations of synoicism, paroicism, and autoicism have been observed in some species. Such a combination is designated as *heteroicism*. When, within a species, the condition of dioecism is combined with any of those above mentioned, such a moss is said to be *polyoicous*. Polyoicism was early known as *polygamy*. Limpricht (1890) and Ruhland (1909) use the same terms but group them in a slightly different way in their classification.

Physiologically there are but two categories of first importance: monoecism, in which the spores, protonemata, and leafy axes (gametophores) are bisexual in their potentialities; and dioecism, the spores, protonemata, and leafy axes being strictly unisexual. In the Bryophytes these terms apply to the gametophyte, whereas in the Spermatophytes the same terms are applied to the sporophyte. Blakeslee (1906) proposed the substitution of the terms *homothallic* and *heterothallic* respectively for *monoecious* and *dioecious* when used in connection with the gametophyte.

There is some confusion in the literature as to what constitutes dioecism (or heterothallism) in the mosses. This situation is partly due to the fact that investigators have depended upon the morphological examinaton of specimens for the determination of their sexual conditions rather than upon cultural studies. Separate axes, one bearing archegonia, the other bearing antheridia exclusively, may have arisen from the same protonema, but this situation is not ordinarily revealed by observation. Some writers have uncritically classed a moss having such separate gametophores as dioecious, regardless of the origin of the gametophores. Thus, Müller (1853, pp. 47–48) says: "On a common stem the inflorescences are monoecious; on different stems they are dioecious." Limpricht (1890, p. 37) agrees with this idea of dioecism and remarks: "So far dioecism of the protonema has not been observed." Ruhland (1909, p. 210) is more emphatic, declaring that in all cases of dioecism both sexes are borne beside one another on the same protonema. Lotsy (1909, pp. 261–262) observes that it is an unsolved question as to whether genuine dioecism occurs in the mosses, since both male and female plants arise from the same protonema, and since in certain so-called instances of dioecism it has been found that sexual organs of one kind may be suppressed for a time by unfavorable environment, but that upon the return of suitable conditions the organs in question appear.

It is evident that the question of genuine dioecism or monoecism in mosses can not be settled by the usual methods of observation. It is necessary to follow the history of single protonemata and their branches from the time of the germination of the spore until the formation of the sex organs.

This method of determining the sexual conditions of certain mosses was first used by É. and É. Marchal (1906). They showed that in *Barbula unguiculata*, *Bryum argenteum*, and *Ceratodon purpureus*, spores produced in individual capsules are heterogeneous and unisexual, some producing protonemata bearing male axes exclusively, others giving rise to protonemata which bear female axes only. Protonemata produced by regeneration from various parts of the gametophyte transmit the sexual characters of the parent plant to the gametophores which they themselves later produce. The sexuality of protonemata thus produced by regeneration, as well as that of protonemata resulting from the germination of spores, can not in any way be influenced by external conditions.

Later the same writers (Marchal and Marchal, 1907) report that protonemata arising aposporously from young sporophytes of such dioecious mosses as *Bryum caespiticium*, *B. argenteum*, *B. fallax*, and *Mnium hornum* bear hermaphroditic axes, a large proportion of male axes, and a very small number of female axes; in other words, bisexual or monoecious gametophytes have been produced in these normally dioecious species. They conclude that the separation of sex potentialities is bound up with the reduction division and that some of the chromosomes carry the sex-determining factors.

M. Wilson (1915) describes organs combining the characters of both sexes occurring on an axis of *Mnium hornum*, a species which the Marchals (1907) had found to be strictly dioecious. Similar exceptional conditions had been reported in the same species and in *Bryum caespiticium* by the Marchals (1909); in *Brachythecium erythrorhizon* by Lindberg (1879); in *Plagiothecium sylvaticum* by Bergevin (1902); in *Atrichum undulatum* by Hy (1884); and in *Mnium cuspidatum* by Holferty (1904). Holding that these deviations from the normal sexual conditions can not be explained by the theory that the separation of sex is tied up with the reduction division, Wilson suggests that sex is determined by certain metabolic processes which are spread over a considerable number of cell generations and which, as a rule, are unaffected by external conditions. The observations of Philibert (1883) and Milde (1865) seem to support his views. The former reported small male plants arising by regeneration from the lower leaves of female plants of *Homalothecium fallax*, *Camptothecium lutescens*, and *Fissidens decipiens*, supposedly dioecious species; the latter author observed bud-like structures enclosing archegonia and antheridia on apparently sterile axes of *Mnium cinclidioides*, which has also been considered to be dioecious. Such unusual sexual conditions may be explained, according to Wilson's theory, by assuming the presence of some unusual factor which has interrupted the normal course of metabolism.

In the non-dioecious mosses it is quite clear that the separation of sexual characters is not related to the reduction division, but takes place, if at all, at some later time in the life of the gametophyte. The Marchals (1909) suggest that in the species for which the denomination *homothallic*, borrowed from the terminology proposed by Blakeslee, would be more exact, the sexual differentiation appears only at the time of the formation of the sex organs.

Strasburger (1910) holds that in monoecious and hermaphroditic mosses the separation of sex takes place at the time of the formation of the sex organs. According to Coulter and Coulter (1918, p. 189), the sexual characters are separated at a time later than that of the reduction division, in connection with some of the vegetative divisions of the bisexual gametophyte. As an example they cite Riccia in which the production of antheridia only takes place for a considerable length of time, but is later succeeded by a period during which archegonia exclusively are formed.

Concerning the sexual conditions in *Funaria hygrometrica* (L.) Schreb., the species especially considered in this paper, various conflicting statements have been made. It has been classed as dioecious by Sachs (1874, p. 368), Goebel (1882, p. 367; 1887, p. 174), Van Tieghem (1891, p. 982), Scott (1904, p. 132), and Bower and Gwynne-Vaughan (1905, pp. 211–212). Campbell (1895, p. 187; 1905, p. 195) says that "Funaria is strictly dioecious"; later, however (Campbell, 1918, p. 622), this statement is corrected and reference is made to Boodle's results.

Bruch, Schimper, and Gümbel (1836–1851, p. 298) described *Funaria hygrometrica* as having a primary stem terminated by a male inflorescence and subsequently giving rise to fertile branches; W. Wilson (1855, p. 268) reported a stem, at first simple and terminated by a barren (male) discoid inflorescence; later branched, the branches bearing terminal fertile inflorescences, the base of the stem and branches rooting. Sullivant (1856, p. 50) described the male inflorescences as borne on innovations. Lesquereux and James (1884, p. 200) reported monoecious, terminal inflorescences, the male borne on the primary shoots, the fertile (female) on the innovations. Limpricht (1890, pp. 198–200) agrees that *Funaria hygrometrica* is monoecious but does not describe the relative positions of the male and female organs. This moss is autoicous, the male inflorescence being borne on short basal branches, according to Braithwaite (1888-1895, pp. 135–136). Dixon and Jameson (1904, p. 300) describe it as autoicous, having a terminal, discoid male inflorescence on a lateral branch; the same writers (1896, p. 276) had previously simply reported this species as autoicous, "the male inflorescence discoid with spreading bracts." Brotherus (1909, p. 421) and Lotsy (1909, p. 9) describe male organs at the apex of a main axis, the female organs on the summit of a lateral branch. These writers all agree that *Funaria hygrometrica* is monoecious, but differ as to whether the male or the female inflorescence respectively is terminal on the main axis or on a lateral branch.

Boodle (1906) reports that in sixty-five specimens out of one hundred two, the female axis was a branch of the male stem. In the remaining specimens different conditions obtained, such as female axes apparently unattached, male axes having no branches, axes bearing no sex organs, and axes attached basally, so that it is impossible to determine which is the main axis and which the branch. From these observations Boodle concludes that *F. hygrometrica* is monoecious, or at least very seldom dioecious.

É. and É. Marchal (1911) state that by experimental methods they have shown *Funaria hygrometrica* to be monoecious. The primary axis terminating in an antheridial head gives rise to the female branch as an innovation. By the death of the upper part of the male axis which has given rise to branches, the branches become separated to such an extent as to be taken for distinct individuals. On such separated axes, branches of one or the other sex may be produced.

Collins (1919) reports the possibility of the origin of a dioecious race of *Funaria hygrometrica* by the regeneration of antheridia and of perigonial leaves from the antheridial head. Gametophytes so produced bore antheridia exclusively and at no time were any sporophytes formed. This suggests that the sexual tendencies are separated in this species before the actual production of the antheridia and archegonia.

Methods

In order to determine the exact sexual condition of *Funaria hygrometrica*, it was considered necessary to make single spore cultures. This was done by first germinating the spores on the surface of a liquid culture medium, and later removing the young protonemata, with the aid of a binocular microscope, to individual pots of soil.

A sufficient supply of spores for the work was insured by bringing gametophores bearing mature sporophytes into the laboratory, and sowing the spores from time to time. It is practicable to do this, since spores of *F. hygrometrica* are viable for several years. The plants were obtained at Eagle Heights, near Madison, Wisconsin.

In order so far as possible to avoid contamination of the cultures, the capsules were thoroughly washed in sterile distilled water. It is well to separate the sporophyte from the gametophyte at the base of the seta, leaving the stalk to be used as a handle for manipulating the sporophyte during the washing of the capsule and the sowing of the spores.

The culture media used in the germination of the spores were tap water, distilled water, and the solution used by the Marchals (1906) in their work on the sexuality of the spores of dioecious mosses. The formula given by them is as follows:

Distilled water	1000 cc.
Ammonium nitrate	1 gm.
Potassium sulphate	0.5 gm.
Magnesium sulphate	0.5 gm.
Calcium sulphate	0.5 gm.
Ammonium phosphate	0.5 gm.
Iron sulphate	0.01 gm.
Potassium hydroxide (10% solution)	a few drops.

The culture fluids were placed in Erlenmeyer flasks and petri dishes and sterilized in the autoclave for from thirty minutes to one hour at five pounds pressure.

Marchal's solution proved the most satisfactory medium. The percentage of spore germination was higher, and a more luxuriant growth of protonemata resulted when spores were sown on this solution than when they were sown on either tap or distilled water. A few cultures were made in which Sphagnum soaked in tap water, distilled water, and in Marchal's solution respectively was used as a substratum for the germinating spores after being sterilized. This proved unsatisfactory because of the difficulty of locating and removing individual gametophytes from the Sphagnum.

Petri dishes were more convenient than Erlenmeyer flasks as containers of the culture fluids because of the ease with which the development of the protonemata could be followed and the individual protonemata could be removed.

The capsules were opened by means of needles sterilized in the flame of a Bunsen burner, and the spores were then scattered on the surface of the culture fluid. In order to make the sowing more uniform, the sporophyte was held a short distance above the medium and the capsule was tapped with a needle. If the spores are allowed to germinate in groups, the protonemata become interlaced and can not well be separated. The cultures were examined daily under a binocular microscope in order to note the time required for spore germination, and the development of rhizoids and branches.

The removal of the individual spores from the petri dishes to soil was attended with some difficulties. The very young protonemata can not be caught up on a needle, so it was necessary to have recourse to the use of a pipette and spore dilutions. Great care must be taken to make certain that but one gametophyte is transferred to each pot. A careful examination of the contents of the pipette, and of the surface of the soil after these contents have been deposited upon it, should be made. The procedure described above was used for the first lot of cultures made. Then it was found that if the gametophytes are allowed to grow until the protonemata are at least seven or eight cells in length, it is comparatively easy to pick them from the culture fluids, under the binocular microscope, by means of a needle.

Three kinds of soil were used for the single spore cultures: a mixture of clay loam and sand; a mixture of leaf mould and sand; and a soil containing a large amount of wood ashes. The last named soil was obtained from a spot where *Funaria hygrometrica* had been growing. The soils were placed in two- and three-inch pots, soaked with Marchal's solution, and sterilized in the autoclave at from ten to fifteen pounds pressure for at least three hours. After cooling, the soils were ready to receive the young protonemata.

Four hundred twenty-five single spore cultures were made at different times from the last of November, 1916, until the last of March, 1917. The cultures were placed in a Wardian case in the greenhouse, where the atmosphere was kept very humid and where the temperature ranged from about 70° in the winter to as high as 112° F. at times during the summer. The pots were set in earthenware plates in which water was kept so that the cultures received water only from below. In this way the danger of contamination by foreign spores and other organisms was minimized.

The plants grew well and by the last of June, 1917, all the pots were covered with gametophores. These cultures have been kept for about two years and all have remained pure, no moss of other species appearing in any of the cultures.

Observations

Germination of Spores and Development of Protonemata

The spores of *Funaria hygrometrica* germinate readily in distilled water, tap water, and Marchal's solution, or on Sphagnum soaked with any one of the three liquids. The cultures must be kept in bright or diffuse light. Room temperature (18° to 21° C.) has been found to be favorable for their germination and growth.

The evidences of the germination of spores are: first, the swelling due to the absorption of water; second, the increase in amount of chlorophyl; and third, the change in the shape of the spores. This change in shape consists in the pushing out of a papillate protrusion from one side of the spore (fig. 1). The exospore ruptures at the apex of this projection, and the protrusion develops into a protonema (fig. 2).

When the protonema has become seven or eight times as long as wide, the first cross wall is formed (fig. 3*a*). The terminal cell increases in length and a second septum appears in it. This procedure continues as the protonema increases in length. Lateral branches may arise from the primary filament while the latter is still very short (fig. 4*a*), or it may remain unbranched for some time (fig. 4*c*). In every instance a branch originates just behind a septum (fig. 4*b*), and the subsequent development of the branch continues in essentially the same manner as that of the primary protonema.

The length of time required for the germination of spores is somewhat variable. When spores are sown on unsterilized Marchal's solution, germination may take place within twenty-four hours; on the other hand, spores sown on the same medium, sterilized, show no evidence of germination in less than about thirty-six hours. In other media the time required for germination varies from three days to one week. In general, spores sown on Marchal's solution germinate more promptly than on the other culture media; but even when sown on the same medium, some cultures require more time for germination than others. Each sowing was made from the spores of a single capsule.

Lesage (1918) finds that the difference in the time required for the germination of moss spores of several species, including *Funaria hygrometrica*, may be attributed to differences in temperature. He finds the optimum temperature for germination to be from 20° to 22° C. At this temperature the spores germinate in thirty-four hours. Schimper (1848) and other writers have noted the period of germination as being from three to five days.

In cultures which I kept in a dark room, no germination could be detected after a period of four weeks. These cultures were then removed from the dark room and placed before a north window, a situation which had been found favorable for germination. When examined later, these cultures showed that a large number of the spores of each culture had germinated. Many spores, however, failed to germinate even when left in the same situation before the window. From my observations it thus appears that the condition of the spores, the culture medium used, and illumination are factors affecting the length of time necessary for the germination of spores of *F. hygrometrica.* Since all the cultures were kept at room temperature, they furnish no information as to the effect of different temperatures on germination.

After the rupture of the exospore and the beginning of the growth of the protonema (fig. 3*b*), or even at the same time, a rhizoid may develop and grow out from the spore or from the basal cell of the protonema in the opposite direction to that of the protonemal filament.

Rhizoids are commonly distinguished from protonemata by their oblique septa, and by the presence of a brown pigment in the cell walls. An examination of the rhizoids shows that chloroplasts may be present in their cells, but not in so large numbers as in the protonemal cells; the septa may be almost perpendicular to the long axis of the rhizoid cells as is the case in protonemata; and the brown color may be almost entirely lacking. These latter conditions are often found in rhizoids arising from the spore and the protonema, as well as in those originating by regeneration from the vegetative parts of the gametophore and from the sex organs.

It was found that protonemata branch profusely in culture solutions, but that gametophoric buds are very seldom formed on protonemata growing in such solutions, although some such cultures were kept from the middle of November until the first week in April. On the other hand, protonemata transferred from these cultures to pots of soil produced leafy axes, on an average, in eight weeks. It might appear that some substance necessary for gametophore development is present in the soil but absent from the culture solution; but since protonemata may be kept in an apparently healthy condition in Marchal's solution for long periods of time, and since gametophores are formed on protonemata growing on filter paper soaked in Marchal's solution, this explanation does not seem adequate. It is possible that the stimulus supplied by the presence of a solid substratum is necessary for the formation of leafy axes, or that if the protonemata had been kept longer in the culture solution such axes would have been formed.

The Distribution of Sexual Characters

After the transfer of the individual protonemata to separate pots of soil, growth takes place for some time. An area varying from one to five or six square centimeters on the surface of the soil in each pot is covered by a green felt-like growth at the expiration of a period of five to six weeks after the transfer. In every case this growth originated from a single spore.

Gametophores do not develop on any of the cultures within a shorter period of time than five weeks after the protonemata are transferred to the soil, and in cultures to which protonemata are transferred when young (two to three weeks after germination) the leafy axes develop in about eight weeks from the time of germination. However, when protonemata are taken from cultures four to six weeks old, the gametophoric buds are formed when the protonemata have been on the soil for from four to six weeks. Thus it appears that the time of transfer of the protonemata affects the time of development of leafy axes. The following are typical instances: For culture 36 the spores were sown December 1, 1916, the protonema was transferred December 12, and the first leafy axes appeared January 28, 1917, approximately eight weeks after germination. For culture 315 the spores were sown on the same date, the protonema was transferred February 10, 1917, and leafy axes appeared April 7, 1917, about four months after germination. For culture 235 the spores were sown December 14 and the protonema was transferred February 2, 1917; leafy axes were observed March 7, 1917, five weeks after transfer and about three months after germination.

The leafy axes arise from the protonemata in a manner similar to that in which protonemal branches originate. A protrusion appears laterally upon the anterior part of a cell just posterior to a septum; intersecting walls are formed, which result in the production of a mass of cells instead of a filament (fig. 5). An apical cell appears when the bud consists of but a few cells. The first leaves are closely appressed to the stem (fig. 6); later they elongate and become folded so as to envelope completely the apical region (fig. 7). Finally the leaves become more or less separated from one another, giving the young gametophore its characteristic bud-like appearance (fig. 8).

The number of gametophores arising directly on individual protonemata is variable, ranging in my cultures from five to twenty-seven. After the development of the gametophores from the protonemata, rhizoids and secondary protonemata are developed from the basal parts of the former, the protonemata arising without previous injury of the gametophores. These new structures in turn give rise to other gametophores from which other rhizoids and protonemata are formed. These processes continue until the surface of each pot is covered by a dense growth of gametophores and protonemata. In every one of my four hundred twenty-five cultures, a large growth of gametophores was secured, and no contaminations by foreign mosses took place in any of the cultures, which remained in good condition for about two years.

Since each pot contains the growth from a single spore, and since this growth consists of several hundred gametophores, the examination of these cultures at the time of the formation of the sex organs should make it possible to determine without a doubt the sexual condition of the species. Boodle (1906) used material obtained in nature, and from this he was able to determine that *F. hygrometrica* was monoecious or "at least seldom dioecious." He added this alternative because he found a number of axes of one or the other sex growing independently, and did not know whether they had originated from the same or from different spores. The Marchals (1911), as pointed out on a preceding page, conclude that *F. hygrometrica* is monoecious, but they do not describe their experiments.

The gametophores remain for some time unbranched and sterile (fig. 9). The first branches observed arose from the basal part of the main axis (fig. 10); in such a case it is often difficult to determine which is the primary axis and which the branch.

The branches resemble the main axis in every detail except that they are usually shorter. The basal part of the branch is enlarged and bulbous, like the basal part of the main axis. The cell walls of the lower part of the stem and of the branch are impregnated with a brown pigment similar to that in the cell walls of the rhizoids. Rhizoids and protonemata arise from the lower parts of both structures. The connection between the branch and the main axis is very loose, and when separated from the axis the innovation may be taken for a separate plant. This condition is probably responsible for the statement often made that *F. hygrometrica* is dioecious, the archegonia being borne on shorter stalks than those which bear the antheridia.

The cultures were examined daily in order to note the first appearance of sex organs. In every case antheridia were developed before archegonia. In the greater number of the cultures, antheridia were observed from one month to six weeks after the appearance of the gametophores. However, in a few cultures they were not seen until six or eight months after the development of leafy axes.

In culture 26, which is typical of the majority of the cultures in the series from 18 to 204, the spores were sown December 1, 1916, transferred to soil December 11, gametophores were noted February 10, and antheridia April 13, 1917. There were several deviations from this program in this series; for example, in culture 29, the history of events is identical with that of culture 26 until the time of the observation of the antheridia, which were first noted December 5, 1917, almost a year from the time of the germination of the spore. In cultures numbered 20, 98, 106, 108, 109, 117, 118, 121, 137, 155, 158, 160, 163, 171, 172, 173, 175, 178, and 182, antheridia were not observed until the latter part of July and the first of August, 1917, about seven months after germination. This difference in the time of production of antheridia in this series can not be attributed to the different soils upon which the cultures were grown, because the difference in the soils used does not correspond with the differences in the history of the cultures. In the series 208 to 234, 235 to 275, 276 to 302, and 303 to 328, there were no noteworthy differences in the history of the development of antheridia.

The antheridial inflorescences may be recognized with the naked eye. The leaves surrounding the group of sex organs are so arranged as to give the head a discoid form (fig. 15). From twenty to thirty antheridia are borne in each group.

The first antheridial inflorescences observed are borne on the summits of unbranched axes (fig. 15), or on the summits of the primary axes (fig. 13) in case the gametophore is branched. As the cultures grew older, examination showed that this location of the male sex organs is by no means the universal one. It is quite common to note antheridia on the apex of an innovation (fig. 12), either archegonia or antheridia being borne on the primary axis. Gametophores with innovations bearing antheridia were noted in twenty-two cultures out of one hundred examined with the object of determining the situation of the antheridia. In each of these cultures from one to ten such gametophores were observed.

From these results it appears that in the majority of instances the antheridia are borne on the apex of the primary axis (figs. 11, 13), or on the apex of the independent, unbranched gametophore (fig. 15); less often are they borne on the innovation, the main axis in such a case bearing either archegonia or antheridia.

The appearance of archegonia was not noted in any of the cultures within less than four weeks after the appearance of antheridia in the same cultures. The archegonial heads are so similar to the sterile heads that dissection is often necessary in order to distinguish them (figs. 11, 13). The antheridial heads are easily distinguished by their discoid shape from the archegonial or sterile heads. From one to five archegonia are formed in a group; three, however, is the most common number observed.

Culture 26 is typical of the sequence of events in the series of cultures from 18 to 204, so far as regards the production of archegonia. The history of this culture has already been given to the time of the appearance of antheridia on April 3, 1917. Archegonia were observed about seven weeks later, on May 30, 1917. In culture 91, antheridia were noted April 26, and archegonia approximately four weeks later, on May 25, the spore having been sown only a week earlier than that from which culture 26 arose. The spore from which culture 20 grew was sown on December 1, 1916, the protonema was transferred December 16, antheridia were observed April 26, 1917, and archegonia not until September 27, 1917. The sequence of events in the majority of the cultures is similar to that in cultures 26 and 91.

In regard to the position of the archegonia, it was found that in the greater number of cases examined, the archegonia are borne on the innovations, the main axis bearing antheridia (figs. 11, 13) or in some instances archegonia. Unbranched axes bearing archegonia were also observed (fig. 14); such axes are more common when the cultures are young.

Considering the results thus far described, it is plain that so far as the material I have worked with is concerned, the gametophytes of *F. hygrometrica* are always potentially bisexual.

All previous writers who have discussed the distribution of the sex organs in *F. hygrometrica* seem to agree that the organs of opposite sex are separated in individual inflorescences. I have been able to find no mention of synoicous inflorescences in this species. The examination of my cultures has revealed the fact that such inflorescences do occur, and in sufficient numbers to warrant their being taken into consideration. In synoicous heads the archegonia may occur singly, or in groups of from two to four. The archegonia are found at the apex of the stem surrounded by antheridia to the number of from fifteen to thirty (fig. 16). Paraphyses of both the filiform and clavate types may be seen scattered among the sex organs in such a head. The filiform paraphyses are restricted to the female inflorescences in the usual distribution of the sex organs, and the clavate forms are found only in male inflorescences. In bisexual groups the clavate paraphyses (*cp*) are found among the antheridia and those of the filiform type (*fp*) only in the proximity of the archegonia. Synoicous inflorescences were observed on primary axes only, or on unbranched gametophores.

These synoicous inflorescences were observed in cultures from the beginning of the period of the production of archegonia until the cultures were about two years old; but none were observed in cultures in which archegonia had not also been formed in their usual positions.

An attempt was made to form some notion of the frequency with which synoicous inflorescences occur in the cultures. The following table shows the range of the percentages of synoicous heads in a few typical cultures.

Culture Number	Number of Gametophores	Number of Synoicous Inflorescences	Percentage of Gametophores Bearing Synoicous Inflorescences
91	51	7	13.72
127	43	3	6.97
166	45	10	22.22
255	28	4	14.28
156	34	4	11.76
Totals	201	28	13.9

The proportion of synoicous axes present in a culture seems to depend upon the age of the culture, a higher percentage being found in cultures about one year old than in those several months of age.

The results thus far described apply to the history of the cultures during their first year of growth. During the second year the axes were examined to note whether any regularity exists as to the sex of the organs borne on later formed branches from the female innovations and on branches of the primary axis, the latter being ordinarily male. As far as could be ascertained, there is no such regularity, since branches of either sex may arise either from the female innovation or from the primary axis.

SUMMARY

1. *Funaria hygrometrica* is strictly monoecious, the spores, protonemata, and gametophores being bisexual in their potentialities.
2. Antheridia always appear earlier than the archegonia.
3. The antheridia are borne in most cases on the apex of the primary

axis of a branched gametophore, a lateral innovation bearing archegonia in the greater number of instances or in some cases antheridia. Antheridia may be borne on unbranched axes.

4. The archegonia are usually found at the apex of an innovation of the male axis; but they may occur at the apex of the principal axis, the innovation bearing either archegonia or antheridia. Unbranched gametophores frequently bear female inflorescences, but they are less common than male inflorescences in this position.

5. Synoicous inflorescences occur in *Funaria hygrometrica.*

6. There is no regularity as to the sex of the organs borne on branches of the archegonial and antheridial axes after the first year of growth.

I wish to express my sincere thanks to Professor C. E. Allen who suggested this work and under whose supervision it was done.

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University of Wisconsin

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EXPLANATION OF PLATE XXXVI

Figures 1–8 were drawn with the aid of an Abbé camera lucida at table level. Leitz oculars and objectives were used: Figs. 1–3 were drawn with ocular no. 4, objective no. 6, tube length 170 mm. (×165); Figs. 4, 6, 7, and 8, with ocular no. 3, objective no. 6, tube length 170 mm. (×650); Fig. 5 with ocular no. 1, objective no. 6, tube length 170 mm. (×390). Figs. 9–15 are enlarged 15 times. The drawings have been reduced one-half in reproduction.

The following abbreviations are used: *r*, rhizoid; *p*, protonema; *s*, spore; *a*, archegonium; *an*, antheridium; *cp*, clavate paraphysis; *fp*, filiform paraphysis.

PLATE XXXVI

FIGS. 1–4. Stages in the germination of spores and development of protonemata.

FIG. 5. Rudiment of leafy shoot.

FIG. 6. Median optical section through older leafy shoot.

FIG. 7. Median optical section through young shoot showing leaves enveloping the growing point.

FIG. 8. Young gametophore.

FIG. 9. Unbranched sterile axis.

FIG. 10. Primary axis having a branch attached basally.

FIG. 11. Primary axis (male) bearing a lateral female branch.

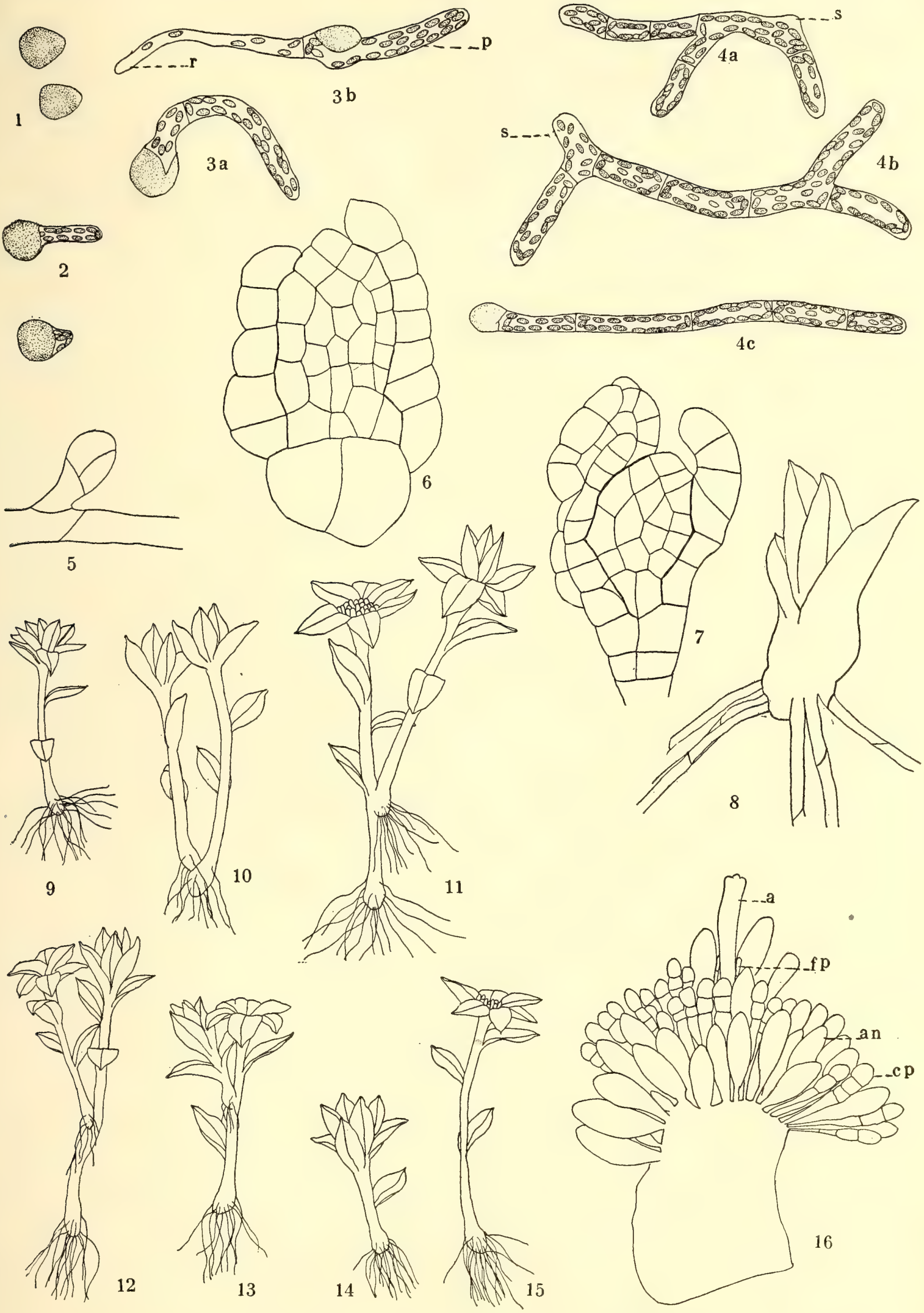
FIG. 12. Primary axis (female) bearing a lateral male branch.

FIG. 13. Primary axis (male) bearing a short female branch.

FIG. 14. Unbranched female gametophore.

FIG. 15. Unbranched male gametophore.

FIG. 16. Synoicous head borne on a primary axis.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Brown: The gametophyte of Funaria hygrometrica.

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AMERICAN JOURNAL OF BOTANY

OFFICIAL PUBLICATION OF THE
BOTANICAL SOCIETY OF AMERICA

...nian Institution
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PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT 41 NORTH QUEEN STREET, LANCASTER, PA.

Entered as second-class matter February 21, 1914, at the post office at Lancaster, Pennsylvania,
under the act of March 3, 1879

AMERICAN JOURNAL OF BOTANY

DEVOTED TO ALL BRANCHES OF BOTANICAL SCIENCE

ESTABLISHED 1914

EDITED BY A COMMITTEE OF THE
BOTANICAL SOCIETY OF AMERICA

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The pages of the Journal are open to members of the Botanical Society of America, or to candidates approved for membership.

Manuscript offered for publication should be typewritten, and should in all cases be submitted to the Editor-in-Chief.

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AMERICAN
JOURNAL OF BOTANY

VOL. VI DECEMBER, 1919 NO. 10

INFLUENCE OF SUGARS ON THE GROWTH OF ALBINO PLANTS

L. KNUDSON AND E. W. LINDSTROM

In the course of investigations on the influence of carbohydrates on plants by the senior writer (1), the occurrence of albino seedlings of timothy was occasionally noted when germinating the seed. The question then arose, What would be the influence of carbohydrates on these plants? Can an albino plant exist and develop when it is entirely dependent on organic material derived externally, as is the case with species of Monotropa and with other non-chlorophyllous plants?

It is true that experiments have been made in which corn and other plants have been grown in the dark with sugars or other organic substances supplied, and that under these conditions plants have maintained themselves for some time and have shown an increase over the original dry weight. The abnormality of the growth in the dark, however, is such, and the augmentation in weight is so slight, that one cannot draw conclusions as to the ability of phanerogamic plants to maintain themselves when the process of photosynthesis is not active.

There is only one way of determining whether or not a phanerogamic plant can develop at the expense of organic matter derived externally, and that is to use albino plants. It might be suggested that one might grow green plants in the entire absence of atmospheric carbon dioxide, but this would not prevent entirely the process of photosynthesis, since the carbon dioxide produced in respiration and retained within the plant would be re-utilized in photosynthesis.

The experiments with albino corn were made possible by the inheritance studies with corn by the junior writer (3), who obtained during his investigations a hybrid that produced albino corn plants in a simple mendelian ratio. The albino seedlings used were all from self-pollinated ears of green plants. The green plants were heterozygous only for the factor that determines the production of white seedlings. No other visible abnormalities were present in this stock. Such stock had been tested against other albinistic seedlings and had always remained free from any tendency to develop plastids and chlorophyll such as other albino seedlings are known to do. In other words, the albino progeny (approximately 25 percent of the total) from the

[The **Journal** for November (6: 357–400) was issued Dec. 18, 1919.]

heterozygous green plants used in the experiment were remarkably uniform in nature.

In addition to determining the growth of albino corn when supplied with carbohydrates, it was believed that the results obtained might be significant with respect to the question whether or not chloroplasts arise *de novo* or arise only from preexisting plastids.

Methods. The plants were grown either in large culture tubes on agar or in water cultures. In the former type of culture the entire plant was maintained in the absence of all microorganisms, while in the water cultures the tops of the plants were exposed to the atmosphere. The seeds were first weighed and then sterilized by the use of calcium hypochlorite (4). For this purpose 10 grams of calcium hypochlorite was added to 140 cc. of tap water, and this solution was shaken for a few minutes and then filtered. The filtrate alone was used for sterilizing the seed. From the sterilizing solution the seeds were transferred directly to the small culture tubes for germination. When the seeds were germinated and it became apparent which seedlings were albino, they were transferred to the large culture tubes or flasks. For the culture tube experiments the procedure was the same as that employed by the senior writer (1), and for the water cultures the method was the same as that used by Knudson and Smith (2).

The nutrient solution used was that of Pfeffer, with the substitution of dibasic phosphate for monobasic phosphate. The solution under the conditions of the experiment caused some inversion of the sucrose, due probably to the interaction between $Ca(NO_3)_2$ and K_2HPO_4 with the production of a small amount of HNO_3.

Experiment 1. In this experiment the influence of sucrose and glucose was to be determined. The plants were grown in large culture tubes 50 cm. x 6 cm., and 200 cc. of the culture solution was used to which was added 1 percent of agar. The concentration of the sugar was 0.10 gram molecular (weight normal), and, for controls, plants were grown in Pfeffer's solution alone. For comparative purposes chlorophyll-bearing plants were also grown, these being grown from seed derived from the same ear that produced the seed yielding albino plants.

The seedlings were transferred to the culture tubes on March 3 and the tubes were then placed in the greenhouse. On April 18 the leaves of one of the albino plants growing in the absence of sugar were dead, but on the remaining albino plants, death of leaves occurred between May 1 and May 4. The duration of the experiment was then approximately 58 days. The results follow in table 1.

Examination of the data reveals the fact that neither glucose nor sucrose permits an increase in dry weight over the original weight of the seed. In fact, there is a decrease in weight, but the decrease is less with sugar than without. The green plants, however, all show a marked increase in weight.

Experiment 2. In this experiment the conditions were essentially the

TABLE 1

Culture Solution	Culture No.	Original Dry Weight (Milligrams)	Total Yield, Dry Weight (Milligrams)	Gain or Loss (Milligrams)
Pfeffer's solution	1	164	92.5	− 71.5
“ “	2	175	98	− 77
“ “	3	180	97	− 83
“ “	4	137	69	− 68
“ “	5*	185	531	+346
Pfeffer's solution + 0.10 mol. sucrose	6	175	125	− 50
“ “ “ “ “	7	177	153	− 24
“ “ “ “ “	8	197	138	− 59
“ “ “ “ “	9*	188	696	+508
Pfeffer's solution + 0.10 mol. glucose	10	171	136	− 35
“ “ “ “ “	11	164	136	− 28
“ “ “ “ “	12	188	158	− 30
“ “ “ “ “	13*	193	1,056	+863

* Green plants.

same as those in experiment 1. In cultures 21 to 25 and 31 to 35, inclusive, the nitrates of calcium and potassium were replaced by the chlorides, and

TABLE 2

Culture Solution	Culture No.	Original Dry Weight of Seed (Milligrams)	Weight of Tops and Roots (Milligrams)
Pfeffer's solution	15	171	25
	16	153	31
	17	162	47
	18	162	49
	19	185	37
	20*	149	217*
Ave. for albino plants			37.8
Pfeffer's solution minus nitrate but with asparagin	21	171	46
	22	163	47
	23	173	32
	24*	165	325*
	25*	138	280*
Ave. for albino plants			41.7
Pfeffer's solution + 0.10 mol. sucrose	26	158	120
	27	162	144
	28	171	86
	29	174	80
	30*	146	615*
Ave. for albino plants			107.5
Pfeffer's solution minus nitrate but with asparagin and sucrose	31	179	110
	32	155	115
	33	180	123
	34	153	97
	35*	162	510*
Ave. for albino plants			111.2

* Green plants.

nitrogen was supplied at the rate of 1.7 grams of asparagin for each 5 liters of culture solution. The seedlings were transplanted to the culture tubes on June 12 and the tubes were placed in the greenhouse. The experiment was concluded on July 30, at which time all the seedlings were dead. Very little difference was noted in the time of death of the seedlings. The data follow in table 2.

In the preceding table the yield is given as dry weight of tops and roots. The residual seed remains were detached from the plant and not included in the weight. It is at once apparent that the addition of sugars makes for an increase in weight, but the substitution of asparagin for nitrates, while permitting equally good growth, apparently does not make conditions more favorable than they are in the solutions with nitrates. As noted by others, asparagin is a favorable source of nitrogen.

Experiment 3. The culture conditions remained similar to those of experiment 1, but the plants were grown in the dark. The data follow in table 3. When supplied with sugars, the plants practically maintained their original weight, while the plant supplied with nutrients alone lost in weight, the loss being about 50 percent of the original dry weight of the seed.

TABLE 3

Culture Solution	Original Weight of Seed (Milligrams)	Total Dry Weight of Plant (Milligrams)	Gain or Loss (Milligrams)
Sucrose 1 percent	133	138	+ 5
Sucrose 1 percent	121	118	− 3
Glucose 1 percent	132	120	−12
Pfeffer's	164	80	−84

Experiment 4. The plants were grown in water culture. For this purpose 500-cc. Erlenmeyer flasks were employed and the volume of solution used was 500 cc. In addition to cultures with sucrose, cultures were also prepared in which a mixture of sugars was used composed of 0.10 mol. glucose, 0.10 mol. sucrose, and 0.01 mol. arabinose. These latter cultures became contaminated near the close of the experiment, but the plants were much like those grown with 0.20 mol. sucrose. The seedlings were transplanted to the culture flasks on March 1 and the experiment was concluded on April 25. The detailed data follow in table 4.

TABLE 4

Culture Solution	Culture No.	Original Dry Weight of Seed (Milligrams)	Dry Weight of Tops (Milligrams)	Dry Weight of Roots (Milligrams)	Dry Weight of Seed Residuum (Milligrams)	Total Dry Weight (Milligrams	Gain or Loss (Milligrams)
Pfeffer's	35	133	45	7	27	79	− 54
Pfeffer's	36	123	54	7	18	79	− 44
Pfeffer's + 0.2 mol. sucrose	37	129	170	60	27	257	+128
Pfeffer's + 0.2 mol. sucrose	38	126	80	50	26	156	+ 30

In this experiment the plants supplied with sugar showed a very appreciable gain in weight, while those grown in Pfeffer's solution alone showed the usual loss. Furthermore, the leaves of albino plants supplied with sugar lived until April 25, while the plants without sugar showed death of leaves on March 25.

General Discussion. In view of the fact that glucose or sucrose is generally considered to be the first sugar product in photosynthesis, it seemed reasonable to expect that the addition of one of these sugars to the culture solution would permit a considerable growth of the albino seedlings. The expectations were, however, in no way realized. An appreciable increase in growth was noted when sugar was available to the plant, and the albino plants supplied with sugar produced from five to seven leaves each, while the check plants possessed only two or three leaves each. Furthermore, in the water-culture experiments the plants supplied with sugar lived about a month longer than did the plants not supplied with sugar. In the tube-culture experiments there was little difference in the duration of life of different cultures.

The difference in length of life between the sugar and the non-sugar cultures in the two types of cultures is explainable in part by the higher concentration of sugar in the water cultures and by the higher temperatures prevailing in the greenhouse at the time when the water-culture experiments were made. In tube cultures, furthermore, the rate of growth is relatively slower than in water cultures.

The failure of albino plants to make a sustained growth and to show marked increase in weight when supplied with sugar is probably explainable by the inability of the plant to absorb sugar rapidly, and in part also by the relatively slow rate of conduction. This hypothesis is strengthened by the fact that even after the leaves are dead the roots may continue to live, and that, if supplied with sugar, the roots may be alive several months after the roots of the check plants are dead. It is possible that if a greater concentration of sugar be used, more beneficial results may be obtained; but certainly it is not possible greatly to exceed the concentration employed in the experiment, which was 0.20 gram molecular.

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VARIATIONS IN PLEURAGE CURVICOLLA (WINT.) KUNTZE

J. L. Weimer

Introduction

The fact that one strain of a fungus may differ considerably from another strain within the same species coming from a different source, growing on a different substratum, or subject to other stimuli, has long been known. Yet the knowledge of the extent of such variations found recorded in literature is scattered and comparatively meager. This lack of exact data on the amount of variation, both morphological and physiological, within the species has led to considerable confusion and often makes it practically impossible to decide whether or not the fungus at hand belongs to a previously described species. It is only by the careful observation and study of a fungus species under the influence of different environmental conditions that its limitations can be determined. Too often fungi are described from specimens from a single source and on but one host or substratum, with the result that some of their characteristics may be overlooked. Later another worker obtains a strain of the same species from another source or under different conditions, notes rather striking differences not mentioned in the original description, and gives it a new name. As a result an organism may receive many names, and only when the group is carefully monographed is it found that these are but variations of one and the same species. The chaotic condition produced by the indiscriminate production of new species was discussed at length at a meeting of the Botanical Society of America in 1908 and the discussion was published in the American Naturalist[1] of the same year.

The purpose of this paper is to record certain variations noted in a strain of *Pleurage curvicolla* with the hope that these may add something to the present knowledge of the extent to which individuals of a single species may vary and yet not afford sufficiently different morphological characters to justify the making of a new species.

Source of the Organism

The strain under discussion first appeared in 1918 as a contamination in a culture of *Sphaeronema fimbriatum* which had been isolated by Dr. L. L. Harter in 1912 from a sweet potato affected with black rot, and which had been in the laboratory continuously since that time. Single ascospore isolations were made, and the organism thus obtained was used in the work here recorded.

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[1] Amer. Nat. **42**: 217–281. 1908.

Variation in the Number of Spores

Perhaps the first and most striking thing observed when a crushed perithecium of this organism is examined microscopically is the large number of spores in the asci. This species as originally described by Winter[2] contains 128 spores in an ascus. Griffiths[3] in his monograph of the North American Sordariaceae states that all but one of his strains, which had 128, had 256 spores in the ascus. He says that he did not count all the spores but counted enough to convince him that there were more than 128, and since another nuclear division would make 256, he assumed that there probably were that number in an ascus. Owing to the great number of spores and to the tenacity with which they cling together, it is impossible to see all the spores, but in the strain under discussion the writer has been able to count 375 in one ascus and 350 in another and in several other instances 300 or more. Counts were made by mounting a single mature ascus on a microscopic slide in a drop of water under a cover slip, and crushing the spores out by pressing on the cover. Nevertheless, it has never been possible to obtain a distribution of the spores which would permit an exact count. In many cases a portion of the spores were counted and the remainder estimated. It was decided finally that there must be in the neighborhood of 500 spores in each ascus and probably 512, which would be the number resulting from another nuclear division after that suggested by Griffiths, or from nine mitotic divisions in all.

It would seem then that *Pleurage curvicolla* may have 128, 256, or 512 spores in the ascus. The number seems to be constant for the strain under all conditions so far as present observations extend.

Spore Size

Perhaps the most reliable characters used in the determination of a species are the spore size, shape, markings, etc. A large number of spores taken from mature perithecia growing on various media have been measured, and the results are tabulated below. Only the dark, opaque spores were considered mature and were measured.

Table 1

Medium	Age of Culture	Receptacle in which Grown	Spore Size Max. and Min. Limits.
Irish potato agar......	30 days	Petri dish	11–13 x 15–19 μ
Irish potato agar......	16 days	Petri dish	10–13 x 13–15 μ
String bean agar.......	14 days	Petri dish	10–13 x 13–15 μ
Corn meal agar.......	15 days	Petri dish	9.5–11.25 x 13–17 μ
Corn meal.............	41 days	Erlenmeyer flask	9–11 x 13–15 μ
Melilotus stem........	33 days	Test tube	9–13 x 13–19 μ
			9–13 x 13–19 μ, max. and min. limits.

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[2] Winter, G. Hedwigia **10**: 161. 1871.

[3] Griffiths, D. Mem. Torrey Club **11**: 1–134. 1901.

It will be seen from the above table that the spore size is rather constant regardless of the medium upon which the fungus grows. The maximum and minimum limits for all the measurements are 9–13 x 13–19 μ compared with 10–11 x 13.5–16 μ as given for *P. curvicolla* by Griffiths, and with 14 μ as given by Winter.

SECONDARY APPENDAGES

The presence of secondary spore appendages has not been satisfactorily demonstrated in this strain. The primary appendage is usually clearly evident on young and recently matured spores, but the examination of thousands of spores in all stages of development has failed to convince the writer of the presence of secondary appendages. However, in the cases of a half dozen spores a small fragment of something which resembled a secondary appendage was noticed at the end of the primary appendage, although none has ever been seen at the opposite end. This may have been the remnant of the secondary appendage, or it may have been some foreign matter or protoplasm from the ascus which had assumed a shape resembling a short appendage. In the instances noted the projections observed were not long and whip-like as described for the appendages of *P. curvicolla*, but it is entirely possible that they had been broken or had deliquesced, since they are said to be "very fugacious."

It seems possible that the presence or absence of these secondary appendages may also depend upon the environmental conditions under which the fungus developed. However, this could be determined with certainty only by long and careful study of many strains of the organism grown under various conditions and subject to different stimuli.

SIZE OF PERITHECIA AND ASCI

It has been seen that the spore size compared very closely with the measurements given for *P. curvicolla*, the maximum and minimum limits including those of the latter. A somewhat greater variation in the size of

TABLE 2

Medium	Age of Culture	Receptacle in which Grown	Perithecial Size Max. and Min. Limits.
Melilotus stems.......	15 days	Test tube	400–572 x 614–786 μ
Corn meal agar.......	15 days	Test tube	529–572 x 815–929 μ
String bean agar.......	14 days	Petri dish	343.2–513 x 472–629 μ
Irish potato agar......	56 days	Petri dish	472–686 x 858–1001 μ
Irish potato agar......	11 days	Petri dish	430–458 x 595–644 μ
Irish potato agar......	30 days	Petri dish	429–543 x 544–829 μ
Corn meal............	41 days	Erlenmeyer flask	200–429 x 743–1101 μ
Melilotus stems.......	33 days	Test tube	400–600 x 715–1004 μ
			200–686 x 472–1101 μ, max. and min. limits
			400–547 x 669–868 μ averages

the perithecia has been found. Perithecia containing mature asci, namely, asci containing dark-colored spores, and growing on different media, were measured. These measurements are given in table 2.

The measurements given for *P. curvicolla* by Griffiths are 350–450 x 550–600 μ, and by Winter, 697 μ high. The measurements tabulated above simply show how great a variation may occur in the size of perithecia. The greatest variation occurred on corn meal on which perithecia as small as 200 μ in diameter were found and some as high as 1101 μ. This is probably due to the quantity of the medium rather than to its quality, since whenever a thick layer of substratum is provided many of the perithecia are submerged and send up long beaks to, or toward, the surface, while when growing on a thinner layer, as in a petri dish, they are usually superficial. Cultures growing in test tubes on Irish potato agar about 3 cm. deep formed perithecia as far as 5 mm. beneath the surface of the medium whose beaks were entirely absent or were short and never reached the surface.

Less attention has been given to the study of the asci, since these enlarge so rapidly when placed in water that the measurements are less reliable. However, measurements of asci taken from a 30-day-old culture on an Irish potato agar plate gave the limits 83–91 x 210–252 μ, as compared with 70–120 x 225–280 μ as given by Griffiths, and 113 μ broad by 257 μ long as given by Winter.

SUMMARY

1. Observations made on a strain of *Pleurage curvicolla* together with records found in literature seem to indicate that this species may have 128, 256, or 512 spores in the ascus, and hence it is assumed that 7, 8, or 9 mitotic divisions probably occur within the ascus.

2. The spores of this strain compare closely with those given for other strains of this species, but there is a somewhat greater variation in the size of the perithecia.

3. Careful study of spores of all ages, both within and outside the ascus, have failed to demonstrate definitely the presence of the secondary appendages which are supposed to be a constant taxonomic character in this species.

INHERITANCE OF SEX IN MERCURIALIS ANNUA

CECIL YAMPOLSKY

The conception that sex is inherited in strictly alternative fashion has been much strengthened by the prevalence in recent years of the Mendelian doctrine that all inheritance is alternative. None the less there has always been abundant evidence among the higher plants of mixed sexuality and of the occurrence of sex intergrades of all degrees. The widespread occurrence of so-called polygamo-dioecism among the flowering plants has been too little considered by the students of the nature of sex and sex determination. The discovery of sex intergrades in animals and the theoretical conceptions drawn from their study by Goldschmidt, Banta, and others is certain to lead to more careful consideration of these well-established facts of mixed sexuality in plants. There is coming to be more and more general agreement that sex characteristics are matters of inheritance in quite the same sense as are other anatomical and physiological characters. The occurrence of dioecious species whose individuals are prevailingly but not absolutely male and female makes it possible to show conclusively that each sex tends to propagate its like and that the doctrine that one or the other of the sexes must be heterozygous for a fixed sex determiner can have no significance for these types at least.

OBSERVATIONS AND DATA

In a preliminary report (1916) a brief summary of the work on my female cultures of *Mercurialis annua* L. was given. This paper deals with female, male, and monoecious cultures of *M. annua.*

As described before, seeds of *M. annua* were collected by Dr. N. L. Britton at Harrington Sound, Bermuda, in September, 1912. From this seed one plant was raised, the original mother plant of my subsequent female cultures. The seed was sown some time in September, 1913, so that when I undertook the problem I had at my disposal a vigorous plant, evidently predominantly female, growing in the propagating house of the New York Botanical Garden. I will designate this as plant *A*. At the time of my first observation the plant was two thirds of a meter in height, vigorous and bushy.

As is well known from the work of Krüger (1908), Bitter (1909), and Strasburger (1909*a*, 1909*b*), which I shall later discuss, *M. annua* produces plants which are purely male and purely female, as well as males and females that produce varying numbers of seeds, the result of the sporadic appearance of sex elements of the opposite sex on the several plants. In my preliminary report (1916) I have indicated that such plants may be regarded as sex intergrades in the light of Goldschmidt's (1916*a*, *b*) results with moths. An examination of tables 1, 3, and 6 of this paper will show the variations in the number of male flowers produced upon the female plants and in the number of female flowers upon the male plants. The number of male flowers found on the female plants varied from 1 to 32, and the number of seeds set on these plants varied from 1 to 230. On a so-called male plant approximately 25,000 male flowers are produced at one time (see page 420). The number of female flowers upon the male plants varied from 1 to 47 and the number of seeds set varied from 1 to 93, while on a so-called female plant thousands of seeds may be produced. Sex gradations may be recognized, starting with the pure female at the one extreme and the plant that produced 230 seeds at the other; the remaining plants are graded between these two. Sex gradations in the male may be noted starting with the pure male as the one extreme and the plant that produced 93 seeds as the other; the remaining plants will grade themselves between these. As described below, I have also had monoecious forms which produce male and female flowers in approximately equal numbers. The species is described as dioecious, occasionally monoecious, by Engler and Prantl (1897).

Plates XXXVIII and XXXIX bring out the characteristic differences in the appearance of the male and female plants of *Mercurialis annua*. The pistillate flowers of the female are clustered in the axils of the leaves, while the staminate flowers of the male are in interrupted spikes which surpass the leaves.

It has been shown by Bitter (1909) that the seed produced upon isolated female plants is due to fertilization by pollen from occasional male flowers produced on the female, and that it is not a parthenogenetic phenomenon. The appearance of male flowers on the female plants is sporadic. They are inconspicuous and difficult to detect. In my work the entire plant was examined at intervals of three days and oftener for the appearance of male flowers.

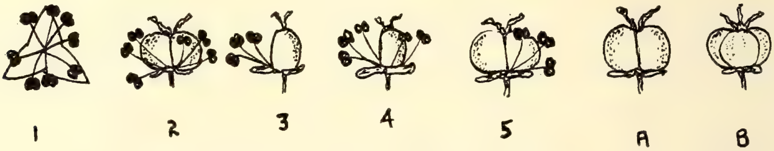
Two months after the observations were begun (in the middle of April), two male flowers were found upon the apices of two side branches. Each male flower bore eight stamens. There was no evidence of a vestigial or aborted ovary. A microscopical examination of the pollen grains of several of the anthers showed them to be plump and to all appearance healthy. An examination twenty-four hours later showed the anthers to be shriveled and about to drop off. In a few days some of the ovaries in the vicinity of the male flowers showed noticeable swelling.

Continued examination of the plant showed variations in the disposition of the male elements. I found five distinct types of arrangement of the pistils and stamens. Normally there is a two-celled ovary which splits at maturity, each cell containing one seed (fig. *A*). Occasionally are found three-carpelled ovaries, each one of the carpels at maturity bearing one seed (fig. *B*).

Figure 1 represents the usual appearance of the male elements on the female plant. The number of stamens ranges from 5 to 16. In such flowers no pistil or trace of a vestigial pistil has been observed. The pollen grains appear plump and healthy.

Figure 2 shows stamens arising around the base of the ovary. The number of stamens varies from 1 to 6 for each ovary. The pollen appears plump and healthy. The pistils of such flowers proved to be functional and produced seed. Figure 5 shows a female flower with 3 stamens.

Figure 3 represents a condition in which one half the flower is male and the other half female. The number of stamens in such cases varies from 3 to 8. The pollen appears healthy and the one-celled ovary functional.



TEXT FIG. 1. Diagrammatic arrangement of pistils and stamens.

Figure 4 represents an arrangement of staminate and pistillate elements essentially like that in figure 3, with the addition of from 1 to 5 stamens at the base of the pistil. The pollen of both groups of anthers appears healthy. The carpel is functional.

Mercurialis annua shows its intersexualism through the appearance on a plant of a given sex of functional organs of the opposite sex. The appearance of flowers of the opposite sex upon a plant is not due to the degeneration of parts as in *Plantago lanceolata* and *Satureja hortensis.* Figure 3, which shows a sectorial arrangement of male and female elements in a flower, illustrates the only case in *Mercurialis annua* in which substitution of floral parts occurs. Sex-intergradation in the female is a matter of the appearance of one or more male flowers or male elements in the manner just described. A comparison of plant no. VII, the female that produced the highest number of male flowers and seeds, and plant no. 247, the male plant that produced the highest number of female flowers and seeds, shows no outward tendency of either one of them to assume the appearance of a male or female respectively. Yet on the basis of sex of flowers produced, no. VII tends towards maleness and no. 247 tends towards femaleness.

It is difficult to determine the proportion in numbers of male to female elements borne on a given plant. Unfertilized female flowers shrivel up within a short time and then fall off. None the less, the sex of a Mercurialis plant can be determined within two weeks after the germination of the seed. The plant blooms from then on to the time of its death. The

TABLE 1 *F_1 Generation from Female Plant*

Plant	1914						1915										Age of Plant at Death	Total Number of Seeds
	May to September				October		February		March		April		May		June			
	♂ Fl.	Seeds	♂ Fl.	Seeds	♂ Fl.	Seeds	♂ Fl.	Seeds	♂ Fl.	Seeds	♂ Fl.	Seeds	♂ Fl.	Seeds	♂ Fl.	Seeds		
I........																	11 months	0
II.......							1			3	1	2					10 "	5
III......					1	3					4	13		17			13 "	33
IV......									2					1			12 "	1
V.......							1		1	7	2	50		30			13 "	87
VI......									1	4		2					12 "	6
VII.....									24	20	6	183	2	27			14 "	230
VIII....														1			12 "	1
IX......	Plant injured and killed																5 "	0
X.......						2			2	15	3	74	3	98			12 "	189
XI......																	6 "	0
XII.....																	12 "	0
XIII....																	10 "	0
XIV.....																	12 "	0
XV.....									2	23	1			8			12 "	31
XVI.....											1	6					12 "	6
XVII....														3			12 "	3
XVIII...																	6 "	0
XIX....																	5 "	0
XX.....																	12 "	0
XXI....									2		4	84	2	23			14 "	107
XXII ...																	11 "	0
XXIII ..										2							12 "	2
XXIV...										1					1	14	13 "	15
XXV....														3			13 "	3
XXVI...																	11 "	0
XXVII..											2	38	1	47			13 "	85
XXVIII.																	7 "	0*
XXIX...	Plant injured and killed																4 "	0
XXX....																	13 "	0
XXXI...									1	5	1	31		11			13 "	47
XXXII..												5		15			12 "	20
XXXIII.																	13 "	0
XXXIV.																	11 "	0
XXXV..	Plant injured and killed																4 "	0
XXXVI.	" " " "																4 "	0
A.......														4			12 "	4
B.......																	11 "	0
C.......	Plant injured and killed																4 "	0
D.......									1		1	2					13 "	2
E.......									1		1	2	1	8			12 "	10
F.......																	11 "	0
G.......												1				4	12 "	5
H.......																	13 "	1
I.......											3			15			12 "	15
J.......									1		2			7			13 "	7
K.......																	12 "	0
L.......									1	2		4	2	30	2	29	13 "	65
M......																	12 "	0
N.......																	12 "	0
Total....					1	5	2		39	82	31	494	19	348	3	47		980

* Vigorous female plant taken to Columbia University greenhouse and pollinated by male—it then set seed profusely. Total male flowers 95.

proportion of male flowers on what I have called prevailingly female plants is probably an insignificant fraction of one percent.

Swelling of the ovary begins a few days after pollination, and ripe fruit appears in a few weeks. 42 seeds were collected during the period in April when stamens appeared on the female plant *A*. The plant then ceased to produce male elements and seed production ceased. Two weeks later stamens again began to appear and seed production followed. 24 more seeds were collected, making the total number of seeds produced by the plant 66. There was no further evidence of male elements. The plant continued to live but with decreasing vigor, the older branches dying off, the younger ones persisting. Female flowers were produced until the very end. The plant was nearly two years old when it died.

F_1 Offspring of the Original Mother Plant

I have reported briefly on the sex of the offspring of this original plant *A*, but will summarize the results here for the sake of completeness. The lots of seed gathered from the mother plant during the month of April, 1914, were sown in pots of previously sterilized soil. In the first lot 36 germinated and in the second lot 14 germinated. Each seedling was potted and labeled and its history was recorded. They were kept in the greenhouse of the propagating house of the New York Botanical Garden throughout the experiment. They were repotted from time to time. As they continued to grow they were placed further apart, so that there was little or no contact of vegetative parts.

The sex of the seedlings, whether they be prevailingly male or prevailingly female, can be determined, as noted, within two or three weeks after germination. The 50 seedlings raised from the 66 seeds were all prevailingly female. The plants were carefully examined at three-day intervals. All the branches of one plant were examined on the same day. With the exception of plants nos. III and X (see table 1), the time of appearance of male flowers and seeds synchronize exactly. In the two exceptions, 3 and 2 seeds respectively were set in October about six months after the seedlings were up. In the case of plant no. III a male flower was seen preceding the development of seed. In plant no. X, although no male flower was seen prior to the setting of the 2 seeds, it is safe to assume that a male flower was developed in their vicinity. As can be seen from the table these plants produced seed later, as did the rest of the plants.

The rather profuse appearance of male flowers and the subsequent development of seeds began in March, and lasted until the end of May.

The stamens in the F_1 plants varied in their number and arrangement in the flowers as was described for the parent plant *A*. In one case the anthers were found to be shriveled and sterile. Plant *D* in March produced a hermaphroditic flower having two stamens, both of which were abortive. The pollen of these two stamens also was shriveled.

24 of the 50 plants produced no seeds. 5 were killed in the process of manipulation. Not one of these lived longer than five months, and consequently it is not known whether they would have produced seeds when older. 3 plants died at the end of five and six months, and had produced no seeds. Plant no. XXVIII, up to seven months of age, bore no staminate flowers nor had it set seeds. When it was placed in contact with a male, after it had been transferred to the Columbia University greenhouses, it bore seeds profusely.

Fifteen plants (see table 1) failed to produce any seeds, although they lived as long as those that set seed. Plant no. VII was the most prolific producer of seeds; it produced 230 seeds, and it also produced the largest number of male flowers—32. Plants nos. X and XXI each produced more than 100 seeds, 189 and 107 respectively. Plants nos. V, XXVII, and L produced 87, 85, and 65 seeds respectively. Plant no. XXXI produced 47 seeds. Plants nos. III and XV produced 33 and 31 seeds respectively. Plant XXXII produced 20 seeds, and of the remaining plants, sixteen in all produced less than 20 seeds each.

The largest total number of seeds for all plants appeared in April, the smallest number in June. The largest total number of male flowers produced was 39. However, 24 of these were produced by plant no. VII. The 26 plants which set seeds produced a total of 980 seeds and a total of 95 male flowers.

Germination of Seeds from F_1 Female Plants

The seeds of plants nos. III and X, which were produced earlier in the season than usual, 3 seeds and 2 seeds respectively, were sown in pots of previously sterilized soil. All germinated, and five plants were developed from them. The rest of the seeds were treated in the same way. The seeds of plants nos. XV and XXIV were not sown. A total of 934 seeds were sown. Table 2 shows the variability in the percentage of germination. In one case, plant no. VII, the germination was over fifty percent. The seeds from twelve plants failed to germinate. From the remaining twelve plants 199 seedlings were secured.

I have previously (*l. c.*) called attention to the low percentage of germination, and I attribute it to the gathering of many of the seeds before they were ripe. The seed capsule upon bursting discharges the seeds. One becomes familiar with the tiny explosions that indicate the bursting of a seed capsule. The force of the explosion is strong enough to send the seeds flying several feet away. To guard against any loss there was a tendency to gather the seeds as soon as they appeared mature, and this resulted undoubtedly in the gathering of many immature seeds; still there may be a tendency here to embryo abortion. 21.3% of the total seeds sown germinated.

TABLE 2. *Germination of Seeds from F_1 Female Plants*

Plant	Number of Seeds	Number Germinated	Percentage	Sex
II	5	0		
III	33	11	33.3	♀
IV	1	0		
V	87	19	21.8	♀
VI	6	2	33.3	♀
VII	230	118	51.3	♀
VIII	1	0		
X	189	15	7.9	♀
XVI*	6	0		
XVII	3	0		
XXI	107	14	13	♀
XXIII	2	0		
XXV	3	1	33.3	♀
XXVII	85	7	9.2	♀
XXXI	47	0		
XXXII	20	0		
A	4	2	50	♀
D	2	0		
E	10	2	20	♀
G	5	0		
H	1	0		
I	15	1	6.6	♀
J	7	0		
L	65	7	10.7	♀
Total	934	199	21.3	♀

* Seeds from plants XV and XXIV not planted.

F_2 Generation from Female Plants

All the seedlings grew to maturity and they were all females.

Thirty-nine plants were kept under observation, and they were treated in the same manner as the F_2 offspring; they were examined throughout their growth. Nineteen plants (see table 3) failed to produce any seeds though they bloomed profusely. Two of the nineteen, however, were injured and killed. One died at six months; the others lived from eight to twelve months. These plants may have been pure females, though they might possibly have produced a few seeds if they had lived longer.

The highest number of seeds produced was 109 by plant no. V_1, a daughter of plant no. V which had produced 87 seeds. However, plants V_2, V_3, V_5, V_7, V_8, and V_9, of the same parentage as V_1, failed to produce any seeds. Plant V_4 produced 10, and plant V_6 39 seeds. The offspring of plant *L* behaved similarly; one produced 10 seeds, the other none.

Plants nos. V_7, $XXVII_1$, and $XXVII_2$, although they showed swollen ovaries, failed to set any seeds and they were counted among those that produced no seeds. They produced no male flowers as far as could be observed. The thirty-nine plants produced a total of 34 male flowers and 358 seeds. The same variations in the disposition of the male elements were observed in these plants as in the other generations.

The 118 plants secured from the 230 seeds sown from plant no. VII were transplanted to the open after they had made sufficient growth. They continued their growth until the end of the season. 27 produced seeds within that time while 91 failed to produce any seeds. They are recorded in table 4.

TABLE 3. *F_2 Generation from Female Plants*

Plant	Date of Planting	1915										1916				Age of Plant at Death	Total Number of Seeds
		March		April		May		June		December		January		February			
		♂ Fl.	Seeds	♂ Fl.	Seeds	♂ Fl.	Seeds	♂ Fl.	Seeds	♂ Fl.	Seeds	♂ Fl.	Seeds	♂ Fl.	Seeds		
III_1	Oct.			2	18	1	8	2	24							8 months	50
III_2	"		2		6											8 "	8
III_3	"			1	7											8 "	7
X_1	"				3											7 "	3
X_2	"			1	9		3									8 "	12
V_1	April									3	46	5	39		24	11 "	109
V_2	"															11 "	0
V_3	"															11 "	0
V_4	"										3	1	7			11 "	10
X_3	"															11 "	0
X_4	"									2	4					10 "	4
X_5	"											2	9		11	11 "	20
X_6	"															8 "	0
X_7	"												1			12 "	1
L_1	"															12 "	0
L_2	"									1	3	1		1	7	12 "	10
III_4	May												5		3	10 "	8
III_5	"									1	11	1	15		17	12 "	43
V_5	"															7 "	0
V_6	"									2	8	3	12	1	19	12 "	39
V_7	"															11 "	0*
V_8	"			Plant injured												5 "	0
XXI_1	"														3	12 "	3
XXI_2	"														2	11 "	2
XXI_3	"															9 "	0
XXI_4	"											1	2			11 "	2
XXI_5	"															6 "	0
XXV_1	"															11 "	0
XXVII_1	"															12 "	0*
XXVII_2	"															11 "	0*
XXVII_3	"												1			12 "	1
A_1	"															6 "	0
A_2	"															10 "	0
E_1	"														1	11 "	1
E_2	"			Plant injured												8 "	0
L_3	June															9 "	0
L_4	"															9 "	0
I_1	"													3	25	11 "	25
V_9	"															11 "	0
Total	"		2	4	43	1	11	2	24	9	75	14	91	5	112		358

* Swollen ovaries; no seed set. Total male flowers 35.

A total of 505 seeds were produced by 20 of the 39 F_2 plants under observation.

TABLE 4. *Germination of Seeds from F_2 Female Plants, Offspring of Plant VII*

Plant	Number of Seeds	Number Germinated	Percentage	Sex
VII$_1$	10	5	50.0	♀
VII$_2$	3	1	33.3	♀
VII$_3$	3	1	33.3	♀
VII$_4$	5	2	40.0	♀
VII$_5$	6	2	33.3	♀
VII$_6$	10	4	40.0	♀
VII$_7$	2	0	—	—
VII$_8$	3	0	—	—
VII$_9$	5	0	—	—
VII$_{10}$	5	1	20	♀
VII$_{11}$	8	3	37.5	♀
VII$_{12}$	7	2	28.6	♀
VII$_{13}$	8	3	37.5	♀
VII$_{14}$	10	4	40	♀
VII$_{15}$	3	0	—	—
VII$_{16}$	8	3	37.5	♀
VII$_{17}$	3	0	—	—
VII$_{18}$	2	0	—	—
VII$_{19}$	2	0	—	—
VII$_{20}$	6	0	—	—
VII$_{21}$	2	1	50.0	♀
VII$_{22}$	5	2	40.0	♀
VII$_{23}$	8	3	37.5	♀
VII$_{24}$	13	4	30.7	♀
VII$_{25}$	3	0	—	—
VII$_{26}$	3	1	33.3	♀
VII$_{27}$	4	0	—	—
Total	147	42	28.2	♀

Germination of Seeds from F_2 Plants

The seeds from plants nos. III$_1$, III$_2$, III$_3$, X$_1$, and X$_2$ were sown shortly after they were collected (see table 3). 11 of the 80 seeds germinated and produced 11 plants. These plants were the first of the F_3 generation of female plants.

Including the seeds from 27 plants, F_2 offspring of plant no. VII (see table 4), 505 seeds were sown. Altogether 104 germinated (see tables 4 and 5), giving an average percentage of germination for the whole generation of 20.1, approximately the same percentage as for the seeds of the F_1 generation.

F_3 Generation from Female Plants

The 11 plants raised from seeds of plants nos. III$_1$, III$_2$, III$_3$, and X$_2$, and the 42 plants of the F_3 generation from plants nos. VII$_1$ to VII$_{27}$ inclusive, were grown to maturity, a total of 53 plants. The other 51 plants were discarded as soon as their sex was determined. *All of the 104 plants were prevailingly female.*

The 53 plants produced 367 seeds. They were not treated like the F_1 and F_2 generations. The seeds as they matured were collected but those from different plants were not kept separate. It was not thought necessary to continue studies on the preceding lines any further. *The 367 seeds produced 118 offspring all of which were prevailingly female.*

F_4 Generation from Female Plants

No effort was made to count the seeds of these plants, of which only a few were kept. The seed secured from them, when germinated, produced as before prevailingly female offspring.

The evidence, it seems to me, is clear that the offspring of selfed female plants tend in the main to be like the parents. It is to be noted that there was marked variation in flower and seed production among them, not occasioned, however, by the influence of pollen from male plants of varying male tendencies since they were all selfed.

TABLE 5. *Germination of Seeds from F_2 Female Plants*

Plant	Number of Seeds	Number Germinated	Percentage	Sex
III_1	50	6	12	♀
III_2	8	3	37·5	♀
III_3	7	1	14·3	♀
X_1	3	0		
X_2	12	1	8.3	♀
V_1	109	21	19.2	♀
V_4	10	0		
X_4	4	0		
X_5	20	2	10	♀
X_7	1	0		
L_1	10	2	20	♀
III_4	8	0		
III_5	43	10	23·2	♀
V_6	39	13	33·3	♀
XXI_1	3	2	66.6	♀
XXI_2	2	0		
XXI_4	2	1	50	♀
XXVII_3	1	0		
E_1	1	0		
I_1	25	0		
Total	358	62	17·3	♀
VII_{15} to VII_{27} (table 4)	147	42	28.6	♀
	505	104	20.1	♀

Male Cultures of Mercurialis annua

In October, 1914, through the courtesy of the Brooklyn Botanic Garden, 12 male plants of *M. annua* were secured. The plants were then growing in beds, and when they were transplanted to pots continued to grow vigorously. They kept on producing pollen. The pollen was viable, as shown by the fact that when they were placed among females seed was set profusely.

The plants were examined at least every three days, and sometimes more often. Male flowers were continually produced. In April, 1915, a female flower appeared on the tip of one of the lateral branches of one of the plants. It was in all respects like the female flowers on the female plant. No other female flowers were produced. Two weeks later the two seeds were collected as they appeared to be mature. The plant began noticeably to lose its vigor, and in the middle of May it died. It had produced two seeds. It is impossible to count the number of male flowers produced during the life of such a plant. Male flowers appear usually three weeks after the germination of the seed. With the increase in the size of the plant there is an increase in the number of male flowers. On a healthy, vigorous, growing male I have computed that over 25,000 male flowers may appear at one time. This I did by stripping a male plant of all its flowers, weighing all of them, and then determining the number of flowers in several one-gram lots. The total weight in grams multiplied by the average number of flowers per gram gives a rough estimate of the number of flowers upon a plant at one time.

A second plant of the twelve behaved in the same way as the first. Two flowers were observed, one with two carpels, the normal condition, the other with only one carpel. The two flowers appeared separately on the ends of lateral branches. The ovaries began to swell shortly after they were discovered, but through a faulty manipulation they fell off before becoming mature. No more female flowers were produced, and the plant died in May. Both the plants were eleven months old at the time of their death. The other 10 male plants failed to produce any female flowers, and by May they had all died.

The 2 seeds collected from the first plant were placed in soil that was previously sterilized. Within nine days one of the seeds had germinated. The other seed proved to be non-viable. Three weeks after germination the sex of the seedling was determined to be male. The plant continued to develop. It lived ten months without producing a single female flower.

Male Cultures from seeds of Female × Male Parentage

100 seeds, presumably from females pollinated by males, secured from the Brooklyn Botanic Garden, were sown in March, 1915. 67 germinated, and of these 36 were males and 31 were females. The male plants were isolated and grown in individual pots. None of the plants was vigorous at any time. Only one of the 36 plants produced female flowers and seeds. In May, two and one half months after sowing, the plant began to develop female flowers. Four such flowers were produced. Three of these flowers bore two carpels each; the fourth had only one. The ovaries began to swell, and two weeks later 7 seeds were collected. The seeds appeared to be weak and sickly. By July all the plants were discarded because of their very poor condition. The 7 seeds failed to germinate.

As was noted earlier, plant no. XXVIII, a seven-months-old female which had produced no seeds, was pollinated by one of the males secured from the Brooklyn Botanic Garden. Several hundred of seeds were collected. These were later germinated and gave approximately a one-to-one ratio of males and females. 167 males from this parentage were potted and grown in the propagating house of the New York Botanical Garden. One plant produced 2 seeds, seven months after germination. The rest of the plants continued to grow vigorously, producing an abundance of male flowers. Some of the males lived twelve months. The 2 seeds failed to germinate.

It was quite evident that the growing of males under greenhouse conditions was not favorable for the production of seeds. Out of a total of 215 males only 4 had produced seeds, giving a total of 17 seeds. From these 17 seeds one male plant was secured. The remaining failed to germinate and 3 seeds were lost. The first two males set seeds when they were ten months old. After that there was a rapid decline of vegetative vigor followed by death. The third male reported as bearing seed did so three months after germination. It is to be noted that the vegetative vigor of that plant was greatly reduced. The fourth case was one in which the male plant produced seeds at the end of seven months, and it continued to grow until it was twelve months old.

In the spring of 1915 seeds were sown out of doors in the experimental plot of the New York Botanical Garden, with a view of watching the male plants throughout the growing season. As soon as the sex of the seedlings was apparent, the females were removed and the males permitted to develop to the maximum. The seedlings were up in May. The plants were examined at almost daily intervals. They made a very vigorous growth, producing countless numbers of male flowers.

The first appearance of female flowers was noticed on the first of August on plant no. 247. The flowers were in various stages of development. During the month of August, 16 other plants produced female flowers and seeds. The relation of male and female elements described for my female cultures does not hold for the male. When the female flowers began to appear, they occurred usually singly among the numerous male flowers on the branches that bore them. As noted before, the female ovary normally is two-celled, producing a two-seeded fruit. Occasionally the female flower on the male plant is one-celled, one carpel only being developed. In the case of the appearance of this condition in the male plant, the missing carpel is not replaced by stamens as is the case when there is a one-carpelled ovary on the female plant, shown in figure 3, page 412. In no instance have I found the female elements on the male plant accompanied by the variations in the disposition of male and female elements which have been noted earlier for female plants and which are represented diagrammatically in the figures on page 412. Upon the male as well as upon the female plant three-seeded fruits (fig. *B*) are found. The female flowers found on a male plant are essentially like those normally found on a female plant. Female flowers when they occur may be found singly on any part of the male plant. Towards the close of the growing season there is a marked tendency for groups of female flowers to appear closely crowded together on branches of certain male plants. Plate XL, figure 1, shows very clearly the grouping of these female flowers in clusters. The female elements never occupy a branch exclusively, but are always associated with male flowers. Sometimes one finds a single female flower on an elongated peduncle which is rather thin and spindly, but which is from fifteen to twenty times as long as the peduncles of the flowers on the female plant. I have found such cases only in the lower and older parts of the male plant.

Plant no. 247 (see table 6) produced the largest number of seeds, 93 in all. This plant differed from the other males in its paler green foliage. The leaves were somewhat smaller and its general habit of growth was sparser, but it showed from the beginning the same general habit as the other male plants. The largest number of flowers and seeds was produced during the month of August, when 68 seeds were collected. During September and October there was a diminution in the number, but this plant continued to bear seeds until it was killed by frost. The seeds throughout the time of their production were found on all parts of the plant, usually occurring in groups of two. Aside from its production of the largest number of seeds (93), there was no reason in its general appearance for placing this plant among the females or monoecious individuals. Approximately 90 other female flowers were produced which, however, failed to develop seeds, and dropped off. Seven plants (see table 6) produced seeds during the month of August and produced no more thereafter, although they continued to grow vigorously, producing countless numbers of male flowers until they were killed by frost in early November. Three plants (see table 6) began to produce seeds in September. Plant no. 253 produced 4 seeds during that month and did not continue to develop any further thereafter, although its vegetative vigor remained unimpaired. The other two produced seeds until they were killed by frost. Nine plants (see table 6) began to produce seeds in October, when the growing season was on the decline. Four plants (nos. 242, 245, 248, 249) began to produce seeds in August. They produced none in September, but again produced seeds in October.

Altogether there were 29 plants that produced seeds, the number of seeds produced varying from 1 to 93. These plants represent, however, about six percent of the total number of male plants under observation, of which there were about 500.

A total of 283 seeds were produced, the highest number, 139, in August, the lowest, 52, in September, and 92 in October. Thirty-one seeds were immature. In a two-seeded ovary one seed often develops faster than the other, so that in gathering the seeds the immature one is likely to be gathered with the mature one. In the act of gathering the seeds nine were lost.

TABLE 6. *Male Plants of Mercurialis annua*

Plant	1915								Age of Plant at Death	Total Number of Seeds
	August		September		October		November			
	♀ Fl.	Seeds	♀ Fl.	Seeds	♀ Fl.	Seeds	♀ Fl.	Seeds		
238	3	5	3	6	3	6			7 months	17 { 1 crushed 2 immature
239	*5	5	3	5					7 “	10 { 1 immature 2 lost
240	3	6							7 “	6 { 1 immature
241	5	10	3	5	2†	5			7 “	20 { 1 immature 3 lost
242	2	3			1	2			7 “	5
243	†1	3							7 “	3
244	3	5							7 “	5 { 1 lost
245	2	4			2	4			7 “	8 { 1 lost 2 immature
247	34	68	7	13	6	12			7 “	93 { 10 immature
248	2	3			2	3			7 “	6 { 2 immature
249	2	4			2	4			7 “	8
250	3	5	1	2	2	4			7 “	11 { 3 immature
252	3	6							7 “	6 { 1 immature 1 lost
253			2	4					7 “	4 { 1 immature
254			4	8	†3	7			7 “	15 { 2 immature
255	2	3	2	3					7 “	3
256	1	2							7 “	2
258	1	2							7 “	2
259	3	5	3	6	3	5			7 “	16 { 5 immature
275					1	1			7 “	1
276					1	2			7 “	2
278			2	3	†3	7			7 “	10
279					11	16			7 “	16‡
277					1	2			7 “	1
280					6	8			7 “	8‡
300					1	1			7 “	1
325					1	1			7 “	1
326					1	1			7 “	1
328					1	1			7 “	1
Total	75	139	28	52	53	92				283

* 2 aborted ovaries.

† Three-parted ovary.

‡ Seeds not used for germination.

Total female flowers, 156.

Germination of Seeds from the Male Plants

In March and April, 1916, 219 of the 283 seeds collected from the male plants reported in table 6 were sown. The seeds of 25 plants germinated (see table 7), while the seeds from two plants did not germinate. The largest number of germinating seeds was 31, those of plant no. 247. Five seeds split their seed coats and showed themselves above the soil, but failed to develop further.

F$_1$ Generation from Male Plants

Soon after the seedlings had established themselves, they were pricked out and placed in individual pots. Three weeks after they germinated the sex of the individual plants became apparent. *The 75 seedlings were all male plants.* The plants as they grew were transferred to larger pots, so as to insure them the maximum growth.

The plants were watched carefully and examined for female flowers. It was thought that perhaps the offspring of plant no. 247 would exhibit the tendency of the parent plant. Not one of the 31 offspring produced a single female flower or a single seed. Toward the middle of September, six months after germination, plant no. 241 produced a female flower, and two weeks later two seeds were collected, one of which was much smaller than the other and appeared undeveloped. Plant no. 244 produced a female flower in the beginning of October, and eleven days later two seeds were collected. In this case, too, one of the seeds was much smaller than the other. Four seeds were collected from the 75 plants. The plants lived from five months to a year. All, however, produced functional pollen, judging from its microscopical appearance.

TABLE 7. *Germination of Seeds from Male Plants*

Plant	Number of Seeds	Number Germinated	Percentage	Sex
238	14*	4	28.7	♂
239	7	3	43.0	♂
240	5	0		
241	16*	4	25.0	♂
242	5	1	20.0	♂
243	3	1	33.3	♂
244	4	1	26.0	♂
245	5	2	40.0	♂
247	83*	31	37.7	♂
248	4	2	50.0	♂
249	8*	2	25.0	♂
250	8	2	25.0	♂
252	4	1	25.0	♂
253	3	0		
254	13*	4	30.7	♂
255	3	1	33.3	♂
256	2	1	50.0	♂
258	2	1	50.0	♂
259	11	1	9.0	♂
275	1	1	100.0	♂
276	2	2	100.0	♂
277	2	1	50.0	♂
278	10	5	50.0	♂
300	1	1	100.0	♂
325	1	1	100.0	♂
326	1	1	100.0	♂
328	1	1	100.0	♂
Total	219	75	34.2	Total

* 1 seed split seed coat but failed to develop further.

Germination of Seeds from F_1 Generation

The two plump seeds and the two undeveloped seeds were sown. Both of the plump seeds germinated and produced seedlings, both of which proved to be males. Both lived to be six months old but they failed to produce any seeds.

The tendency of males to produce males is obvious. Still the fact that they can produce a few pistils sporadically and thus set seeds, makes it equally clear that sex determination is not absolute. Some plants are pure males, others set a few seeds. There are all grades of maleness as of femaleness.

Table 8. *Summary of Results of Male Cultures*

Number of Plants	Number Setting Seeds	Number of Seeds	Number Without Seeds	Number of Seeds Germinated	Sex
12........	2	8 (3 lost)	10	1	♂
36........	1	7	35	0	
167........	1	2	166	0	
500........	29	283	471	75	♂
75........	2	4	73	2	♂
790........	35	304	755	78	

Monoecious Mercurialis annua

It has already been stated that monoecious forms of *M. annua* have been described in systematic works (Engler and Prantl, 1897). Krüger (1908, p. 338) reports the appearance of monoecious individuals among his cultures. Bitter (1909, p. 124) observed monoecious forms. The twelve individuals that Bitter describes as occurring in the old Botanical Garden in Berlin had large male spikes. The monoecious forms I have been working with showed no such male characters.

From the mixed seeds secured from the Brooklyn Botanic Garden I obtained a number of plants that, although in their appearance they were like the females, yet in the production of seeds showed a marked contrast. Seed production in these plants began about three months after germination. Before this time the plants produced an abundance of female flowers which dropped off shortly after their appearance upon the plant. The plants were examined at intervals for the presence of male flowers. Four plants were kept under observation. A large number of male flowers was produced daily after their first appearance. I counted over 100 male flowers on one plant at one time. My work on the monoecious plants has not gone far enough to warrant drawing final conclusions, yet it is safe to assume that in the amount of seed produced there will be fluctuations similar to those in the female and male cultures. There are approximately as many male flowers as female flowers upon the monoecious individuals. The four plants produced seeds about the same time. The following is the record of seeds collected from the four on the same day.

Plant	
1.	320 seeds
2.	100 "
3.	269 "
4.	725 "

Three weeks after germination the seedlings, which represented about fifteen percent of the seed sown, produced female flowers. No male plants were produced. I am not ready to report on the subsequent behavior of the seedlings.

Discussion

Sex Determination and the Alternation of Generations

In the discussion of the time at which sex is determined in plants it must be borne in mind that there are two generations to be considered, the gametophytic haploid and sporophytic diploid generations. This alternation of generations occurs regularly (except in plants that reproduce parthenogenetically only), so that a consideration of sex determination must take both generations into account. This makes possible the existence of sex differentiation at two distinct stages in the life history. In the seed plants, which we have been considering, sex differentiations are always present in the gametophytes (female, embryo-sac, and male, pollen tube), while the sporophyte may be of any grade from male through hermaphrodite to female. From the standpoint of the sex of the gametophyte, if we attempt to assume a segregation of sex determiners in the reduction division, we have the curious condition that the already sex-determined individuals may give rise to sporophytes of graded sexes ranging from males through hermaphrodites to females. The assumption, therefore, must be made that after fusion a redetermination occurs at one or several stages which brings about the graded sex forms.

In the dioecious liverworts, where antithetic alternation of generations is conspicuous, sex determination may occur at the reduction division. No one has yet observed sex differentiation in the sporophyte. Strasburger (1909*a*) finds in Sphaerocarpos, in which the spores remain united in tetrads, that in the vast majority of cases, two spores of the tetrad give rise to female prothallia and two spores give rise to male prothallia.

Allen (1917) in a brief note reports the presence of sex chromosomes in the gametophyte of Sphaerocarpos. Although it is difficult to count the chromosomes, he says that there are eight. In the female gametophyte one of the chromosomes is larger and thicker than the rest, while in the male gametophyte there is one that is smaller than the other seven. This is a case in which the differential chromosome condition is found in the gametophyte, while in animals it is, of course, associated with the diploid generation. The sporophyte of Sphaerocarpos is more or less globular in shape, the foot being in the form of a bulb. We must assume that the sporophyte contains the double number of chromosomes, namely 16, two of which are the larger one of the female and the smaller one of the male. The significance of these two chromosomes is not yet understood, especially since the sporophyte of Sphaerocarpos is not sexually differentiated.

It is interesting to note that sex determination in the monoecious liverworts has no relation to the reduction division, as appears to be the case in Sphaerocarpos. The spore that produces the gametophyte in such a form has the potentiality of producing both sexes, both antheridia and archegonia being found on the same thallus.

That ordinarily dioecious liverworts exhibit mixed sexuality in the gametophyte has been observed in a few isolated cases. Taylor (1837) describes an androgynous gametophore in *Dumortiera irrigua.* Townsend (1899) found in *Preissia commutata* the male thallus bearing archegonia. Ernst (1907) found androgynous receptacles in *Dumortiera velutina.* Cutting (1910) found that an archegoniophore of *Marchantia polymorpha* bore antheridia. Limpricht (1890) observed transitional structures between antheridia and archegonia in *Jungermannia Kaurini* and *Cephalozia Gottschei.* Although the cases studied are few, fixity of sex or fixity of sex organs is apparently not absolute in the liverworts. The literature is, however, very meager upon this subject.

Sex determination in the dioecious mosses may also occur at the reduction division, that is at the division which forms tetrads. In the monoecious mosses no segregation of sex determiners at the reduction division can be conceived. There are a few cases among the dioecious mosses in which mixed sexuality occurs. Müller (1848) found in *Leucobryum giganteum* the archegonia transformed into branchlets and no paraphyses present, while the perichaetium was excessively developed. Antheridia occurred on these female plants. The species is described as dioecious. Ruthe (1874) found that *Physcomitrium eurystoma* has three kinds of shoots, male, female, and hermaphroditic. He also found antheridia at the base of the archegonia in female shoots. Philibert (1883) found in *Homalothecium fallax, Campothecium lutescens*, and *Fissidens bryoides* that protonemata derived from dying leaves and parts of female plants produced small male plants. These mosses are described as being dioecious. Bergevin (1902) found mixed sex organs in *Plagiothecium sylvaticum.* He shows figures of archegonia transformed into antheridia, all stages in the transition being observed. His figures show a condition in which the archegonium can be recognized in part of the mixed structure, the other part being transformed into an antheridium. Wilson (1915) found in *Mnium hornum* normal antheridia, bisexual organs, and modified archegonia on the same axis. The number of chromosomes was haploid, and it was not a case similar to that described by the Marchals (1907).

In the prothallia of the homosporous ferns the succession in the appearance of antheridia and archegonia is modifiable by very many external conditions. According to Wuist (1913), the gametophyte of *Onoclea struthiopteris* is normally dioecious. A monoecious condition can be induced by transferring the prothallia from one culture to another. If this be true, sex in such forms is determined at the reduction division but modifiable afterwards.

In the heterosporous ferns, as in the seed plants, the sex of the gametophyte is determined before the reduction division. Thus in Salvinia the sori are differentiated as to sex in the sporophytic generation. In the Marsileaceae the differentiation is not one of sori but of sporangia. This is not absolute, because microsporangia have been induced experimentally to produce macrospores and macrosporangia, microspores. Shattuck (1910) has been able experimentally to arrest the formation of the macrospores and microspores in *Marsilea quadrifolia*. He did this by subjecting young sporangia to unfavorable conditions for growth, such as reduction in light, and by means of cold water spray. Occasionally he found that when microspores were thus retarded, macrospore-like cells were produced. He also found microspores in macrosporangia.

In both dioecious and monoecious seed plants the sex of the gametophyte is fixed. However, in so-called perfect flowers the sex organs as they develop are transmutable. The sex of the gametophyte generation is prevailingly determined before the reduction division; thus as a rule the macrosporophyll will bear the macrospore, the microsporophyll the microspore; but pistillody of the stamens and staminody of the pistils have been repeatedly observed, that is, a macrosporophyll may be transmuted into a microsporophyll or *vice versa*. When reduction occurs the sex of the gametophyte has already been fixed.

While morphologically the gametophyte generations in the flowering plants appear to be unalterable, so that a macrogametophyte produces only eggs and a microgametophyte only male gametes, it has been urged that in dioecious phanerogamic plants none the less the gametes of either one of the gametophytes may be of two kinds, male-determining and female-determining. The cytological investigations in animals in which either the male or the female may produce two kinds of gametes, as evidenced by their nuclear constitution, have been made the basis of views favoring the existence of a similar condition in plants. If we briefly examine the process whereby a female gametophyte is produced, we shall find it difficult to reconcile the assumption that there are two kinds of eggs, male and female, without bringing in accessory hypotheses. If we assume that the homosporous ferns are the ancestors of the seed plants, we note a distinct specialization in the gametophytes of the seed plants as contrasted with the ferns. The gametophyte of the homosporous fern bears both sperms and eggs. The gametophyte is capable of producing both gametes but both are distinct, the sperm a motile gamete, the egg a stationary gamete. In the seed plants the gametophytes may be conceived as having developed from a monoecious gametophyte of the fern through the suppression of one or the other of the gametophytes, thus a macrogametophyte through the suppression of the microgametes of a gametophyte and a microgametophyte through the suppression of the macrogametes of a gametophyte. Such a development leaves no room for the assumption that there are either two kinds of eggs as Bateson (1909) claims for the female *Bryonia dioica*, or two kinds of sperms as Correns (1907) claims for the male *Bryonia dioica*. The eggs of the homosporous fern cannot be assumed to be of two kinds, because the resultant sporophyte is not sexually differentiated, or at best it is potentially hermaphroditic. Nor, for the same reason, can the sperms be conceived as being of two kinds.

The results obtained in selfed *Mercurialis annua* females show that, although there may be gradations in strength of femaleness, no male plants are ever produced, which, on Bateson's assumption, should be possible. It is to be noted that Bateson has drawn the conclusion from his crosses of *Bryonia dioica* × *B. alba* that the eggs of the female of *B. dioica* are of two kinds, male-producing and female-producing, and that this accounts for the approximately one-to-one ratio of the sexes in a population of males and females of *B. dioica*. Mercurialis females, if their eggs are of two kinds, male-producing and female-producing, should yield both male and female offspring on Bateson's assumption. The offspring of selfed female plants of Mercurialis are, however, all female or prevailingly female.

Male gametophytes arise from microspores. These microspores have their sex determined before the reduction division. The male gametes are produced by the male gametophyte which is the haploid generation. If there is to be a differentiation of the male gametes into those bearing male determiners and those bearing female determiners, it must occur within the microgametophyte—that is, after the reduction division has occurred, through either the loss or the addition of cellular material, unless it be assumed that the whole gametophyte generation is of two kinds, male-determining and female-determining. This process is not at all analogous to the production of male gametes in the males of animals. If we assume with Correns (1907) that there are two kinds of pollen grains, male-producing and female-producing, we must assume that the differentiation occurred at the reduction division. Yet we find that pollen grains are male haploid generations resulting from male spores. Neither Bateson nor Correns has adequately discussed the relation of the gametophytic generations which, in plants, are interpolated between the spore and the gametes, to problems of sex determination. The results obtained in male cultures of *Mercurialis annua* negative Correns' assumption of two kinds of pollen grains. Correns like Bateson arrived at his conclusions from the results of crosses of *Bryonia dioica* × *B. alba*. According to Correns, the presence of two kinds of pollen grains, male-producing and female-producing, in a dioecious form such as *Bryonia dioica* accounts for the approximately one-to-one ratio in a population of males and females. On that assumption, selfed males of *Mercurialis annua* should yield both male and female offspring. However, the offspring of selfed males of *Mercurialis annua* are male or prevailingly male, and thus negative Correns' assumption of the two kinds of pollen grains. Further, because of the occurrence in plants of one species of hermaphrodites, mixed sexes, and intersexes, as well as separate male and female plants, it is difficult to conceive of any exact quantitative separation of gametes such as is being postulated in animals.

If there is segregation in the reduction division of sex determiners which are carried through the gametophytes, sex in the sporophyte would be determined at the fusion of the gametes, therefore syngamically determined. The work of Correns, which I shall later discuss, suggests, however, that sex may be determined before fertilization in the case of gynomonoecious forms when a form of more pronounced tendency comes together with one of a weaker tendency.

Theories of Sex Inheritance and Sex Ratios

It is the present tendency for investigators of animals and plants to assume that in dioecious species, whether of animals or plants, one or the other of the sexes is virtually a hybrid, that is, it is heterogametic. When the heterogametic individual mates with the homogametic individual, recessive for sex, there results a one-to-one ratio of sexes. The dioecious organism is assumed to have either two kinds of eggs and one kind of sperm, or one kind of egg and two kinds of sperms.

The behavior of the sex chromosomes in animals fits well with the contentions of the investigators who claim that sex is inherited in the ordinary Mendelian fashion. To be sure, the number of animal forms that have been shown to exhibit this quantitative difference in their sex cells is so far relatively small, yet an increasing number of forms is constantly being added to the list.

The common observation that in dioecious forms there is approximately a one-to-one ratio in the proportion of the sexes lends itself very readily to the analysis of sex on the basis of Mendelian formulae. The work of Morgan (1909) and von Baehr (1909) on phylloxerans and aphids, in which all the fertilized eggs produce females, because of the production of sperms from the spermatocytes of which only those with the X-element are functional, demonstrates in these cases a selective mortality of sperms (or spermatocytes) of one kind. Upon fertilization the homogametic eggs are fertilized by only one kind of sperm, and this explains the appearance of one kind of individual to the exclusion of the other.

On these theories, sex is determined syngamically at the union of the two gametes. If the male is heterozygous for the sex factor, a female arises when the egg unites with a female-determining sperm, a male when the egg unites with a male-determining sperm. If the female is heterozygous for the sex factor, a female arises when a female-determining egg unites with any sperm, a male when a male-determining egg unites with any sperm. This syngamic determination of sex should exclude any possibility of sex alteration prior to fertilization or after fertilization. It is to be noted that the sex chromosome theory does not attempt to explain how plants with so-called perfect—hermaphroditic—flowers are produced, and, in contrast with the animal kingdom, these include the great bulk of the seed plants. I shall discuss this aspect of the question later.

Cytological evidence for sex chromosomes in phanerogamic plants is so far negative. Sykes (1909) failed to find any difference in the nuclei of male and female plants of Hydrocharis, Morus, *Bryonia dioica*, *Lychnis dioica*, *Mercurialis perennis*, Sagittaria, and *Cucurbita Pepo*. Strasburger (1910) and Malte (1910) found no evidence of chromosomal differences in male and female cells of *Mercurialis annua*.

The evidence that a phanerogamic plant may produce two kinds of eggs or two kinds of sperms is only of an indirect character. It has been based mainly on the work of Correns (1907) and Bateson (1909), who worked with crosses between *Bryonia dioica* and *B. alba*, to which I have referred in an earlier paper (1916). Correns assumes that there are two kinds of pollen grains; Bateson assumes that there are two kinds of eggs. According to both assumptions one of the sexes is heteroyzgous for the sex factor. Shull (1910), working on crosses of *Lychnis dioica* with "hemaphrodite mutants," claims to verify Correns' assumption that the male is the heterozygous sex.

Sex ratios in dioecious forms have been reported in the main as conforming to the Mendelian hypothesis of sex. In mass populations the expected one-to-one ratio is secured with slight fluctuations in one or the other direction. Heyer (1884) found a ratio of 100 males to 114.93 females in 40,000 hemp plants and 106 males to 100 females in 21,000 *Mercurialis annua* plants. Fisch (1887) found a ratio of 100 males to 154.23 females in 66,327 hemp plants. Strasburger (1900) found a ratio of 100 males to 128.16 females in 10,662 plants of *Melandrium album*. But in the offspring of certain matings in both the animal and plant kingdoms there are striking exceptions to the one-to-one ratio. Certain matings will give exclusively female or almost all female offspring, whereas other matings will give the opposite result. Doncaster (1916) reports for the gall-fly, *Neuroterus lenticularis*, that galls from six females produced 4,235 males and 83 females, and six other galls produced 5,139 females and 117 males. Montgomery (1908) secured an overwhelming number of males as contrasted with females in a culture of the Aranead *Lutroductus nactans*, namely, 37,210 males to 4,539 females. Doncaster (1913) found in his cultures of the moth *Abraxas grossulariata* lines of females that tended to produce only female offspring. He also had bisexual lines in which the proportion of males to females was about equal. However, in mating some of the unisexual lines he secured offspring that were all female.

Riddle (1917), on the basis of a number of sex ratios reported by various authors showing that the degree of closeness of relationship between the parents affects the sex ratio, concludes that family crosses yield only male offspring, 20 males to 0 females; generic crosses a ratio of 4.9 males to 1 female; specific crosses 4.3 males to 1 female; racial crosses 1.9 males to 1 female. The normal sex ratio for any of these species when they are crossed *inter se* is 1:1 or at most not higher than 1.3 males to 1 female. Riddle confirms Whitman's conception that width of cross in doves and pigeons is of first importance in determining sex ratios. In general, the wider the cross the higher is the proportion of males. The proportion of males to females reported by Riddle ranges from 15 males to 1 female to as low as .78 male to 1 female.

The true relation of the sexes in plants is made specially clear by the occurrence of the variously graded forms, sex intergrades, that are found between the pure female and pure male extremes as I have described them. The sporadic appearance of male flowers on the female plants has been established for hemp and Lychnis, as well as for Mercurialis.

Shull (1914) in a cross between his broad and narrow-leaved forms of *Lychnis dioica* secured 96 plants, 95 of which bloomed and proved to be all females. In matings between F_2 narrow-leaved males and numerous females he secured a total of 2741 males and 14 females. An hermaphroditic specimen when used as pollen parent produced 276 females.

The Mendelian hypothesis of sex in itself cannot account for the preponderance of one sex to the exclusion of the other. Other ancillary hypotheses must be assumed, such as selective fertility of sperm or egg, selective viability, and lethal factors, in order to explain the results.

Inheritance of Femaleness

The history of the work on the inheritance of sex in Mercurialis is of interest. Krüger (1908) reported that what he thought were parthenogenetically produced seeds on female *M. annua* plants, upon germination gave only female plants and thus confirmed Strasburger's observation. Bitter (1909) first showed that seed production on female plants of *M. annua* is due to the presence of isolated male flowers on the female plants. His work showed definately that in this case the seeds do not arise parthenogenetically. Bouche (1881) had observed the appearance of isolated male flowers on female plants of *M. annua.*

Bitter (1909) began his studies in 1903. In a series of experiments he obtained a total of 723 female to 21 male plants from a number of parents which he calls females. However, it is to be noted that not all the plants were grown in sterilized soil, and as we cannot be certain that no seed from males was present, the females in all probability were not all pure females—in one case he describes one as having "many male flowers," and we have no indication as to whether the males were growing near by and from what plants the females were pollinated.

Strasburger (1909*a*) reports on his female cultures of *M. annua* and confirms Bitter. He secured 907 seeds from his female plants. These he sowed. 148 germinated, only 16.3 percent, and all were females. He also made a cytological examination of the developing embryo and concluded that fertilization had occurred. Malte (1910), who undertook a detailed cytological study of the plant, found the same to be the case.

The original mother plant that I worked with produced 66 seeds, 50 of which germinated, giving rise to female plants. The behavior of the F_1 plants (table 1) shows great fluctuations in the number of seeds produced. Only six F_1 plants, offspring of the mother plant, produced an equal or larger number of seeds, the range being from 65 to 230 seeds. The remaining 44 plants produced fewer, the highest number being 47, the lowest number 1. There was no marked tendency for the offspring to resemble the parent in seed production. With the exception of plants nos. III and X, there is an obvious synchronism in the period of male flower production and seed development (see table 1).

The F_2 generation females (table 3) show the same marked variation in seed production as the F_1 generation. The total seed production was much lower than for the F_1 generation. A total of 358 seeds was produced by 20 of the 39 plants grown, whereas in the F_1 generation 980 seeds were produced by 26 plants of the 50. The offspring of plant no. VII in no instance showed the tendency to profuse seed production which characterized the parent (table 4).

Altogether, including the seeds of the F_1, F_2, and F_3 generations, 1918 seeds were sown, producing 471 offspring all of which were female.

Bitter (*l.c.*) noted that small insects, such as ants and plant lice, are attracted to the female flowers by the two nectaries that are present. Weiss (1906) made this observation earlier on the same form. Strasburger (1909*a*) estimates one thousand pollen grains for each anther, so that the possibility for the pollination of many female flowers by one male flower is great. I found that by keeping the plants separate, so that no parts touched, there was little danger that insects would spread the pollen from one plant to another. On the other hand, I crowded a group of my F_3 female plants closely together and they set seed profusely. They were permitted to sow themselves on the greenhouse bench, and as soon as the seedlings showed their sex they were destroyed. No attempt was made to count the seeds, but I was able to count 8,155 seedlings all of which were females.

Inheritance of Maleness

De Vries (1903), in the second volume of *Die Mutationstheorie*, has a drawing of a male branch of *Mercurialis annua* bearing fruit. However, he does not say anything about the behavior of the seed on germination. It was Strasburger (1910) who first reported upon the germination of the seeds set on male plants. Among the male cultures under his direct observation 15 plants bore seed, the numbers of seeds ranging from 3 to 8. He had a total of 74 seeds from male plants. Not all, however, were ripe, and a number were discarded. From the remainder he raised 36 plants to maturity all of which were males.

The behavior of the male plants under my observation confirms Strasburger's observations. The seeds from the male plants produced males. From 304 seeds sown 78 plants were secured, all of which were males. It is to be noted that only 35 plants out of almost 800 produced seed. Table 6 shows the variation in seed production.

In comparing table 1 with table 6, one is struck with the difference in seed production between females and males. It must be borne in mind, however, that a comparison on the basis of seed production is not justifiable. It was previously pointed out that an anther may contain 1000 pollen grains. Assuming that all the grains are functional and that only one anther is produced upon a female plant, it is theoretically possible that 500 of the two-celled ovaries may be pollinated and produce seeds. A vigorous female may at one time produce thousands of flowers. The maximum number of seeds secured from a female flower upon a male plant is three when a three-celled ovary is present. Inasmuch as the number of female flowers upon a male plant is small, only a few seeds are secured. It would perhaps be more accurate to compare the total male flower production on females with the total female flower production on males. Here too one meets with the difficulty of finding all the male flowers on a female plant because they are so inconspicuous, whereas all the female flowers soon develop seed upon a male and are readily detected. The 26 female plants (table 1) produced at least 95 male flowers (it is not claimed that this number includes all that were produced). The 29 male plants (table 6) produced 156 female flowers, and to these should perhaps be added 90 that dropped from plant no. 247.

Nevertheless, it should be noted that fewer male than female plants produced seeds. The females may also, in some cases, have set seed from stray pollen from other female plants in the vicinity that produced sporadic male flowers.

If we leave out of account the sporadic male flowers, the selfed females of *Mercurialis annua* may be said to have recorded their own "gametic constitution" by producing only prevailingly female offspring. The work of Krüger, Bitter, and Strasburger, as well as the present results, do not confirm Bateson's (*l.c.*) assumption that the female bears two kinds of eggs, male-producing and female-producing. On Bateson's assumption, selfed females should produce both male and female progeny.

Leaving out of account the sporadic female flowers, the selfed males of *Mercurialis annua* may be said to record their own "gametic constitution" by producing only prevailingly male offspring. Strasburger's and the present work do not confirm Correns' assumption that the male bears two kinds of pollen grains, male-producing and female-producing. On Correns' assumption male plants should produce both male and female progeny.

The existence of these sporadic male and female flowers on plants predominantly of the other sex shows, however, that the conception of sex as determined by hard and fast unit factors is probably radically wrong. A truer view of the nature of sex as a fluctuating variant is given by the recognition of the existence of these sex intergrades.

My results tend to bear out the well known breeding law that "like tends to beget like." This is true of sex as of other characteristics.

I have not yet pollinated female flowers on males with pollen from male flowers borne on females. It would be interesting to know the nature of the offspring from seed so secured. The difficulties of manipulation are very great. It is almost impossible to be certain that a female flower, appearing upon a male, is at any time free from some of the thousands of pollen grains that are continually being shed. I hope some time to be able to publish results on crosses between female intergrades and male intergrades, female intergrades and pure males, pure females and male intergrades, as well as on all possible crosses between hermaphrodites and graded males and females.

Correns (1913), in discussing Strasburger's results with *Mercurialis annua*, mentions work of his own with *Valeriana dioica* which yielded similar results. Females pollinated by males gave males and females, the latter in excess. Some of the males bore hermaphroditic flowers which were self-fertile and the seeds with one questionable exception produced only males.

Tournois (1914) reports securing two female plants from seeds set on male hops (*Humulus lupulus*). The results, however, seem so incomplete and the data so few that they must be further investigated.

The fluctuation in the proportion of males and females reported for dioecious plants may in part be explained on the basis that in a given population one or the other sex may be producing selfed seed. Heyer (*l. c.*) found a ratio of 100 males to 114.93 females in 40,000 hemp plants. This ratio may be explained on the assumption that among his plants there were female plants which produced male flowers and that selfed seeds resulted which in turn gave rise to female offspring. At the same time normal pollination by the males occurred and the progeny from that seed would occur in a one-to-one ratio. The addition of the selfed seed from female parents would raise the proportion of females in the population. When the proportion of males is greater than that of the females, it may be assumed that the male parents produced selfed seed in addition to the seed set by the females through normal pollination. Heyer (*l. c.*) in 21,000 *Mercurialis annua* plants reports a ratio of 106 males to 100 females. However, the above suggested assumptions do not entirely explain deviations from the expected one-to-one ratio as judged from an analysis of sex ratios in experimental plants and animals.

Inheritance of Sex in So-called Polygamous Species, and Sex Intergrades

The inheritance of sex in species that exhibit this mixed distribution of the sex elements (so-called polygamous species) has been studied in a number of forms with results that are as yet by no means clear. I shall summarize the most important results in the literature in so far as they bear directly on the question of sex inheritance.

Darwin (1889) in his discussion of dioecious and polygamous plants cites the case of *Thymus vulgaris*, a gynodioecious plant, occurring in two forms, females with well developed ovaries and with stamens much reduced and functionless, and hermaphrodites with functional ovaries and stamens. The female produces seed when fertilized by the pollen of the hermaphrodite, and the hermaphrodite is self-fertile. Seeds from the female and the hermaphrodite produce offspring of both sexes.

Echium vulgare is gynodioecious. Darwin (1889) found that there are intermediate forms between females and hermaphrodites. The intermediate forms are in all respects like the females, with the exception that one or two of the stamens produce perfect anthers while the rest of the stamens are rudimentary. Of 23 seedlings raised from seed from the hermaphrodite, 1 was intermediate, the other 22 were hermaphrodites.

Correns has made an exhaustive study of inheritance in certain polygamous and gynodioecious forms. We are indebted to his wide researches in that direction for much of our knowledge of the behavior in inheritance of such forms. Correns (1904) reports for *Satureja hortensis* three classes of individuals:

1. Gynomonoecious, with normal hermaphroditic flowers, hermaphroditic flowers with shriveled anthers, and female flowers.
2. Gynomonoecious but functionally female, with hermaphroditic flowers having shriveled anthers and female flowers.
3. With female flowers only.

He did not find individuals bearing only hermaphroditic flowers. The results show that the offspring of the female plants are almost exclusively female, the offspring of the hermaphroditic and gynodioecious plants at least 2/3 hermaphroditic (combining classes 1 and 2) and 1/3 female.

Silene inflata occurs in five forms: male, andromonoecious, hermaphroditic, gynomonoecious, and female plants. The females and hermaphrodites are in excess. Correns (1904), working with the two latter forms, found that the hermaphrodite produced exclusively hermaphrodites, and that the females, when pollinated by the hermaphrodite, produced almost exclusively females. According to Correns, the female is dominant over the hermaphroditic tendency.

In a later paper, Correns (1905) describes six classes of individuals in *Satureja hortensis*:

1. Plants with normal hermaphroditic flowers.

2. Plants with normal hermaphroditic flowers, and hermaphroditic flowers with shriveled anthers.

3. Plants with hermaphroditic flowers with shriveled anthers.

4. Plants with normal hermaphroditic flowers, hermaphroditic flowers with shriveled anthers, and female flowers.

5. Plants with hermaphroditic flowers with shriveled anthers, and female flowers.

6. Plants with female flowers.

Some plants which he studied throughout their growth, in the beginning showed only hermaphroditic flowers, then passed through the stages noted in the classes above, and finally became female plants. These apparent females explain, as he thinks, why he secured females in selfed hermaphroditic cultures in 1903. Correns (1905) explains that in his first cultures he had examined the offspring at the end of the season and had not taken into account the changes which had taken place during the growing season. The 134 females of his hermaphroditic culture were probably hermaphrodites. They produced 252 hermaphrodites and 24 females. Correns (1906) finds essentially the same results for Satureja, *Silene inflata*, and *S. dichotoma*. In *Plantago lanceolata* the different forms reproduce their like quite well. The author here reiterates the two laws that he had formulated as a result of his experiments, namely; (1) Each sex form produces germ cells which have the tendency to produce the same form, and (2) The tendency of the phylogenetically younger, *i.e.*, those forms which have become unisexual, dominates over the hermaphroditic tendency. Raunkiaer (1906), a little earlier in the same year, reported on the inheritance of forms in two gynodioecious species, *Knautia arvensis* and *Thymus vulgaris*. In Knautia four hermaphrodites produced 80 offspring, 73 hermaphrodites and 7 females. Six females produced 272 offspring, of which 44 were hermaphrodites, 197 females, and 31 gynomonoecious forms. Correns (1906) calls attention to the fact that two of Raunkiaer's females were really gynomonoecious forms and became female at the time of the experiment. In Thymus, female plants produced 44 offspring, 42 of which were females and 2 hermaphrodites. 60 offspring of hermaphrodites included 21 hermaphrodites and 39 females. Correns explains this result by claiming that those plants which Raunkiaer considered females in his offspring were probably gynomonoecious individuals.

Correns (1908) reports further on the influence of the male germ cells on the sex of the individual. Plantago shows many intermediate forms between the female and the hermaphrodite. Plants whose sex had been determined the previous year were crossed. Thus plant no. 122, female with small anther rudiments (sex constant for 3 years), was crossed with the hermaphrodite no. 149 and gave 89.9 percent female offspring, the rest showing various gradations between hermaphroditic and female. Plant no. 122, female, crossed with plant no. 118, hermaphrodite, gave 97 percent females. Plant no. 124*b*, female with small anther rudiments (1906 showed hermaphroditic tendency, 1907 and 1908 female), when pollinated by hermaphroditic plants nos. 149 and 118 respectively, gave 73.8 percent and 81 percent females, the remaining offspring being distributed among forms between hermaphrodite and female. Plant no. 128, which showed both female and hermaphroditic tendencies in 1906 and 1907, and which appeared to be female in 1908, when crossed with the hermaphroditic plants nos. 149 and 118 gave 18.5 percent and 58.8 percent females respectively.

From the above noted results, Correns concludes that the egg is responsible for the nature of the sex of the offspring. Plants nos. 122, 124*b*, and 128 when pollinated by plant no. 118 gave varying percentages of female offspring. The pollen also has an influence on the nature of the offspring. When no. 118 was used as pollen parent more pure females were produced than with no. 149 as pollen parent. The more pronounced the production of sex cells with a female tendency is in a plant, the less influence the pollen parent has on the offspring. Plants nos. 122 and 124*b* which are strong females gave with plants nos. 149 and 118, 89 percent and 97 percent females. Plant no. 128, which was only partly a true female, gave only 58.8 percent female offspring when pollinated with plant no. 118. It may be conceived that if the female plants were capable of producing functional pollen capable of self-pollination, the offspring therefrom would be prevailingly female and would thus behave like the selfed females of *Mercurialis annua.*

Shull (1910) describes the appearance of hermaphroditic mutants among his pure bred families of *Lychnis dioica* L. The hermaphrodites were of two kinds. When his so-designated "*A*" and "*B*" hermaphrodites were used as pollen parents, there resulted 398 females, 305 hermaphrodites, and 2 males. When his so-designated "*C*" and "*D*" hermaphrodites were used as pollen parents, there resulted 65 females and 73 males. Hermaphrodite *A* as female parent was pollinated by a normal male and there resulted 21 hermaphrodites, 2 females, and 11 males. Shull assumes that plant *A* is a modified male and thus explains his results. When *A* was self-pollinated it produced 23 females and 25 hermaphrodites. *B* upon self-pollination gave 110 females and 95 hermaphrodites. The author harmonizes his results with those of Correns on the crosses between *Bryonia dioica* and *B. alba.* The male is the heterozygous sex. The hermaphrodite must have eggs of one kind and pollen grains of two kinds.

The existence of hermaphrodites in *Lychnis dioica,* although described as a pathological phenomenon, the result of the invasion of the smut *Ustilago violacea* in the female of the species, has been first described by Shull as a normal condition. An analysis of the behavior of hermaphroditic plants *A* and *B* makes it appear that these two plants were graded females. When they were used as pollen parents and when they were selfed they produced hermaphroditic and female offspring, which would suggest such a condition.

The hermaphroditic offspring may be considered as graded females. When *A* was used as a female parent and pollinated by a male the resultant offspring may be regarded as graded females; they were, according to Shull, hermaphrodites and males. Shull's work does not seem to strengthen Correns' assumption that the pollen in a dioecious form is of two kinds.

In crosses between female *Fragaria virginiana* and male *F. chiloensis*, Richardson (1914) secured 16 female, 12 male, and 6 hermaphroditic offspring. From another cross he secured 49 females, 27 males, and 16 hermaphrodites. In a cross between female *F. virginiana* and hermaphroditic *F. grandiflora* as male parent, he secured 20 females, 0 males, and 14 hermaphrodites. In a cross between female *F. virginiana* and hermaphroditic *F. visca*, he secured a few fertile males, very few fertile hermaphrodites, sterile females, and some apparent males. A cross of the nearly pure male *F. chiloensis* (hermaphrodite) and *F. grandiflora* (hermaphrodite) used as the male parent, gave a minority of hermaphrodites, a majority of males, and no females.

I have discussed the relation of the foregoing data and theoretical considerations to the general question of sex intergradation in another paper which is to appear soon.

Summary

1. Female plants of *Mercurialis annua* show gradations in degree of femaleness from pure females, which have no male flowers and never set seed when isolated, to those which produce a considerable number of male flowers and seeds.

2. Offspring of selfed plants which are prevailingly female are female and prevailingly female, showing similar variations in the number of male flowers and seeds produced to those displayed by the parent plants.

3. Male plants of *M. annua* show gradations in maleness due to the sporadic appearance of female flowers and the subsequent production of seed on the male plants.

4. Offspring of prevailingly male plants are male or prevailingly male.

5. While the tendency for females to produce females and for males to produce males is obvious, the sporadic occurrence in varying numbers of flowers of the opposite sex on either form makes it clear that sex intergradation is a condition to be recognized in the plant kingdom. The rather sporadic appearance of flowers of the opposite sex upon a plant points also to the fact that sex determination is not absolute.

6. The condition of sex intergradation points to the fact that a theory of inheritance of sex which assumes fixed sex factors segregated at the time of the reduction division, cannot account for the production of sex intergrades.

7. In a discussion of sex determination in plants in which alternation of generations regularly occurs, both generations must be borne in mind.

An examination of the literature brings out the fact that the sex of either one of the generations in cryptogamic and phanerogamic plants is not fixed.

8. Results in inheritance of polygamous species tend to bear out the conception that in such forms there are gametes of graded potencies, this being true of the egg as well as of the male gamete. This, together with the behavior of selfed females and males as in *Mercurialis annua*, may explain fluctuations in the expected one-to-one ratio.

The work reported in the foregoing pages was begun in 1914 under the direction of Professor R. A. Harper. The writer wishes to express his indebtedness to Professor Harper for his suggestions and criticisms during the pursuance of this study.

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EXPLANATION OF PLATES XXXVII—XL

Plate XXXVII

Fig. 1. Male plant of *Mercurialis annua* 3 weeks old.

Fig. 2. Female plant of *M. annua* 3 weeks old.

Fig. 3. Male plant of *M. annua* 6 weeks old.

Fig. 4. Female plant of *M. annua* 6 weeks old.

Fig. 5. Branch of monoecious individual showing the frequency of distribution of male and female flowers: male flowers at *a*, female flowers at *b*. The anther sacs have discharged their pollen.

Fig. 6. Branch of female individual showing the presence of one male flower and many female flowers: *a*, male flower; *b*, female flower.

Fig. 7. Branch of female individual showing the presence of two male flowers, female flowers, and maturing seed: *a*, male flower; *b*, female flower; *c*, maturing seed.

Plate XXXVIII

Female plants 3 months old; seed set by pollination from male.

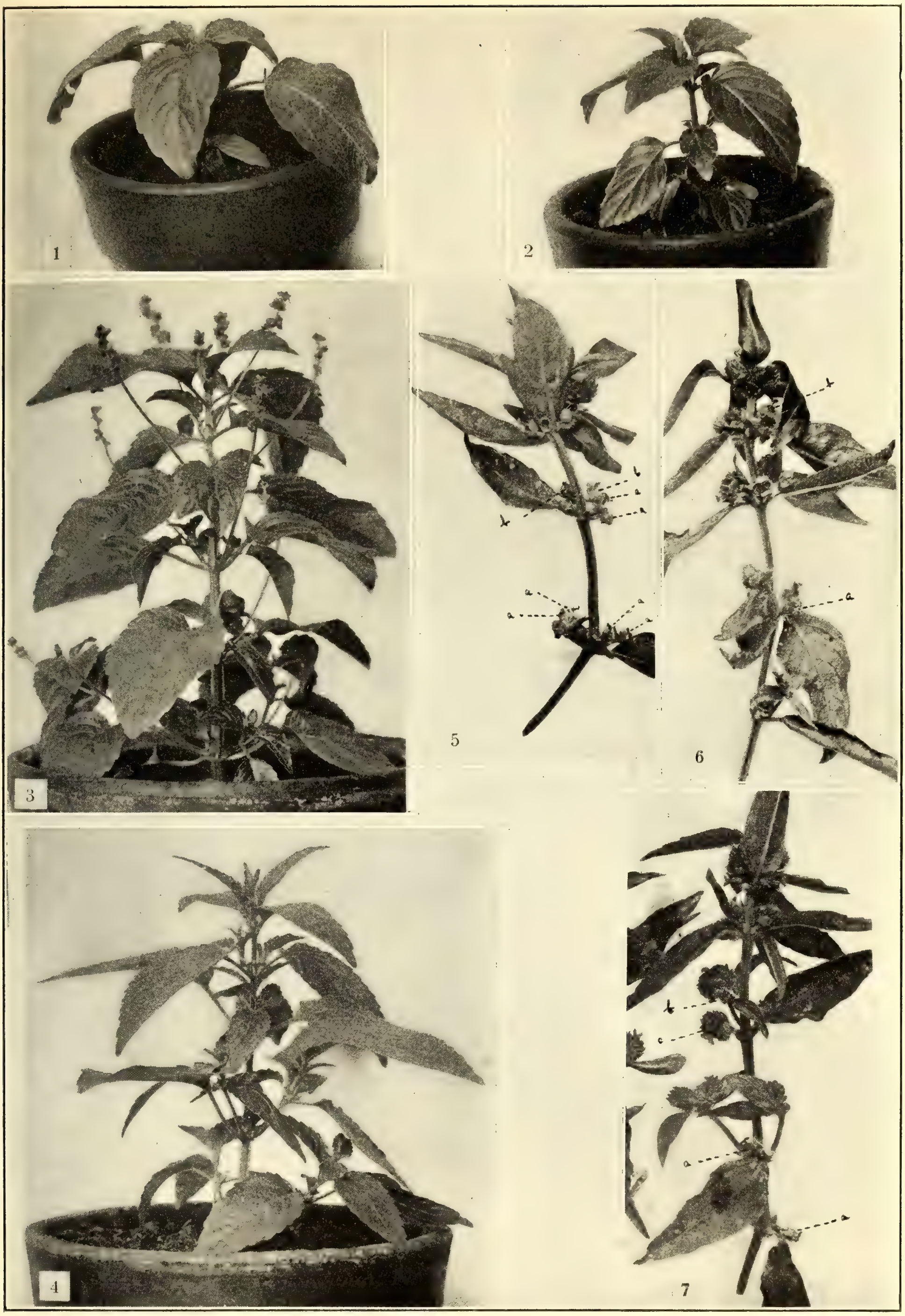
Plate XXXIX

Male plants 3 months old, grown from same series of seed as female.

Plate XL

Fig. 1. Branch of male plant no. 279, showing appearance of seed on male branches.

Fig. 2. Branch of male plant no. 280, showing appearance of seed on male branches.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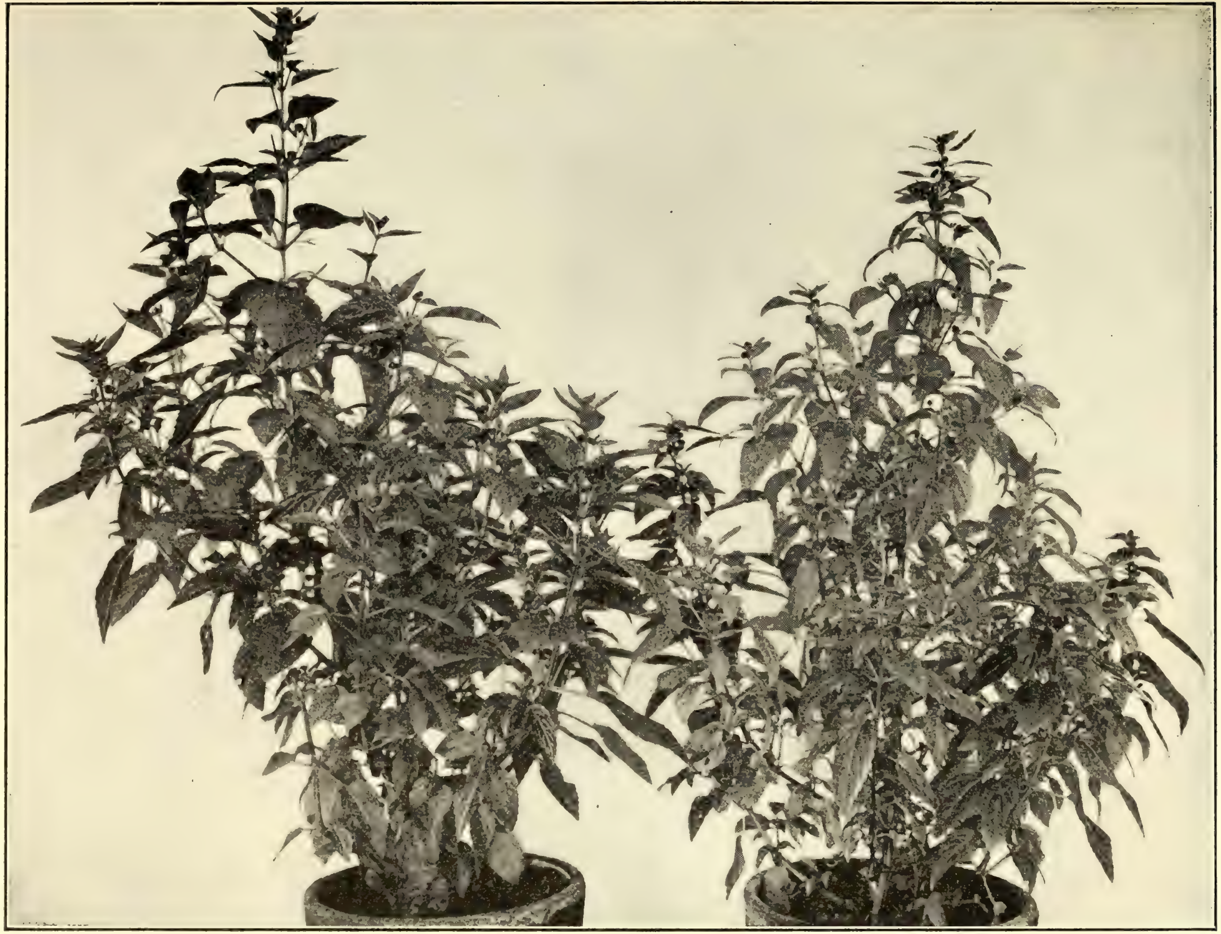
YAMPOLSKY: SEX IN MERCURIALIS.



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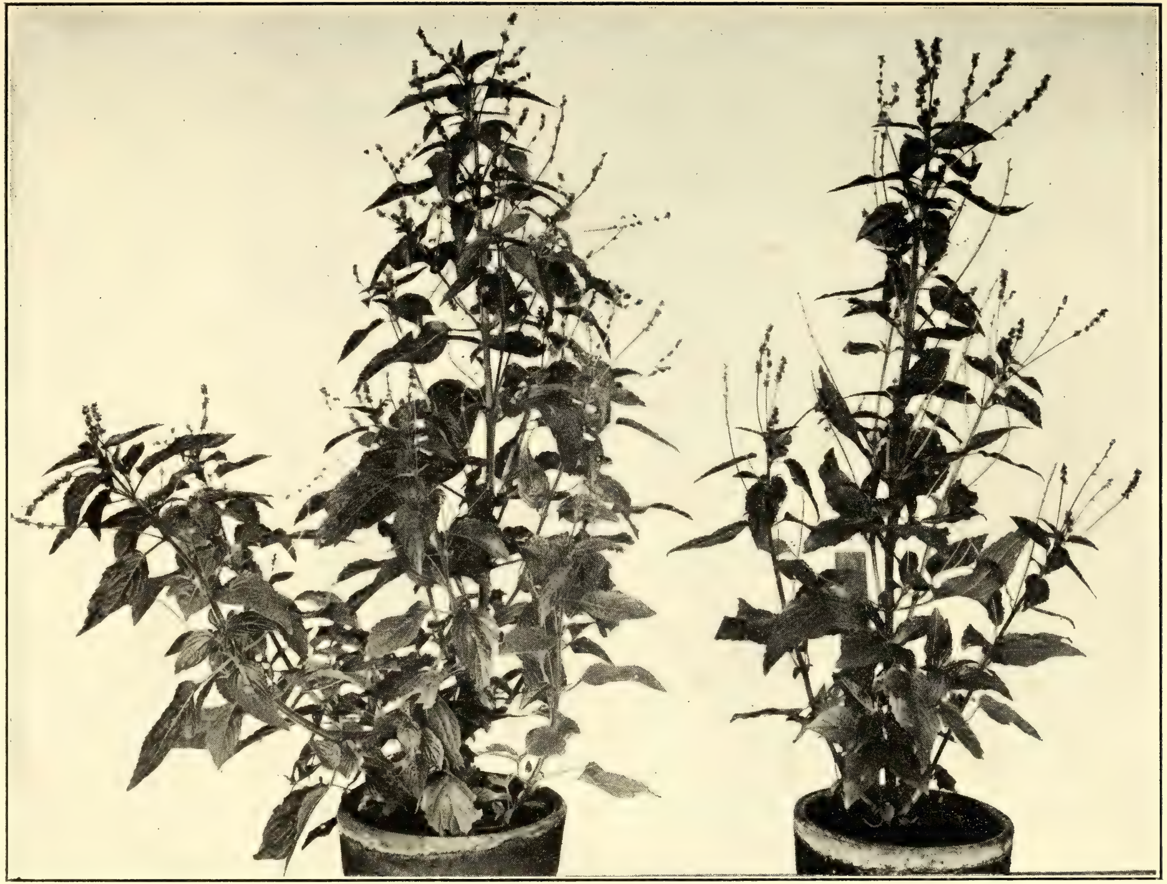

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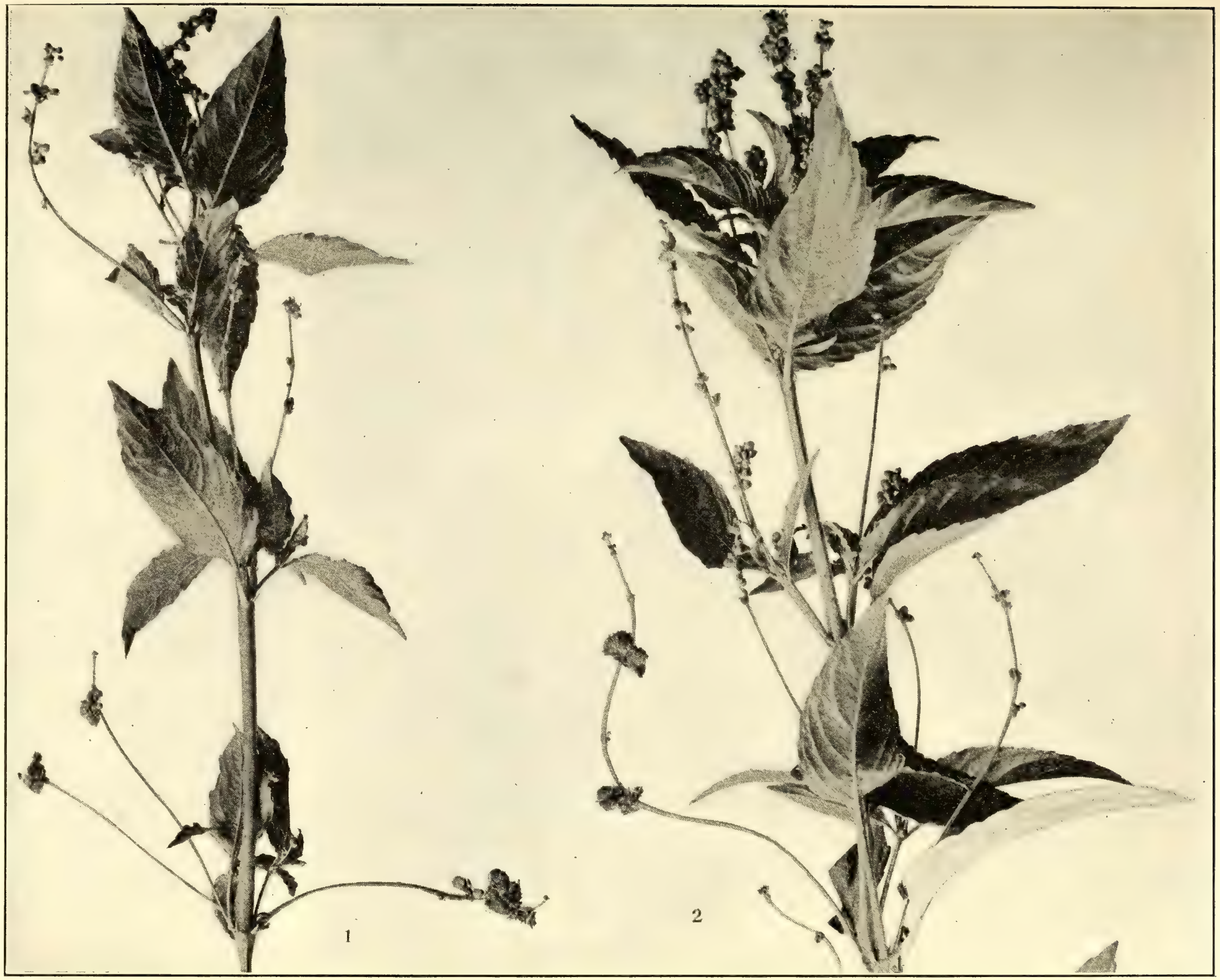

YAMPOLSKY: SEX IN MERCURIALIS.

GERMINATION AND FURTHER DEVELOPMENT OF THE EMBRYO OF *ZEA MAYS* SEPARATED FROM THE ENDOSPERM[1]

Demetrius Ion Andronescu

The Problem

The problem which the author proposes to investigate is a threefold one: (1) To ascertain the germination and further development of embryos separated from endosperms and also from scutella; (2) To find a proper medium for the germination of embryos which will furnish a substitute for the removed endosperms and scutella; and (3) To ascertain the effect in heredity, if any, of this traumatism.

Historical

Bonnet (2), as quoted by Stingl (12), is credited with being the first to study the germination of embryos detached from the cotyledons in his work with *Phaseolus multiflorus*.

Sachs (8), working also with *Phaseolus multiflorus* and with *Zea Mays*, pointed out that embryos when separated from their endosperms germinate poorly. He stated also in his *Physiology of Plants* (page 373) that the embryo during germination has a parasitic relation towards its endosperm, digesting and sucking up the latter through its absorption organ, the scutellum.

Gris (6) germinated embryos of *Mirabilis jalapa*, *Zea Mays*, and many other plants in moist sponges. He observed that the first stage of germination was based on the nutritive material found in the embryo itself, and only after the roots of maize attained a length of 3 cm. was he able to observe the appearance of sugar in the endosperm; he believed the scutellum to be only an absorption organ of the embryo.

Van Tieghem (13), working with seeds of *Helianthus annuus*, *Mirabilis jalapa*, and *Zea Mays* to ascertain (1) the dependence of the various parts of different organs of the embryo, and (2) the dependence of the embryo upon the endosperm; found that the plumule, radicle, and cotyledon, when separated and in a proper medium, but not the scutellum of the maize seed, are capable of independent growth and even of regeneration. Further, he found that the embryo of Mirabilis germinates easily without its endosperm, although the later is necessary in the advanced stage of germination for the development of the plumule. He demonstrated that the endosperm can be replaced by a paste made of triturated endosperm of the same seed or of seeds of different species, or by merely a paste made of starch to which have been added mineral nitrates and phosphates. He claimed therefore that the nutritive power of the endosperm does not depend upon its cellular organization. In another series of experiments, in which were involved *Mirabilis longiflora*, *Canna aurantiaca*, *Aucuba japonica*, and *Phoenix dactylifera*, the same author (14) concluded that "The endosperm of oleaginous and aleuronic seeds has a probe activity; it digests itself. . . . The starchy and cellulosic endosperms are, on the contrary, passive; they are digested by the embryos. . . . "

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[1] This article was read by title at the thirteenth annual meeting of the Botanical Society of America at Baltimore, Md., December 26–28, 1918. Owing to various circumstances the writer has been unable, up to the present time, to continue his investigations on this subject, but he has decided to publish these meager observations hoping that they may be of interest to others whose work is in the same or similar lines.

Bolciszwesky in 1876, as quoted by Brown and Morris (3), experimenting with embryos of oats, rye, and lupine which he germinated in moist soil, also concluded that the endosperm is not necessary to germination.

Brown and Morris (3) raised the question: "Is there any residual life in the cells of the endosperm?" By applying, or, to use the authors' own term, by "grafting" new living embryos to seeds of barley which had been killed by the action of heat, chloroform, and alcohol, these embryos developed almost normally, using these dead endosperms. These observations supplement the statement of Sachs that "The endosperm of the grasses is a dead storehouse of reserve material; therefore during the germinating period the life of the embryo is not parasitic but saprophytic."

Stingl (12) found that the endosperms of the seeds of wheat, rye, barley, and oats in many cases may be substituted one for another during the process of germination.

Dubard and Urbain (5) germinated embryos of seeds of barley, oats, and maize, without endosperm and cotyledon, in Knop's 0.5 percent nutrient solution. Some of their conclusions were: that the endosperm is not indispensable for the development of plants; that the endosperm is favorable in the first stage of germination; that the embryo possesses in itself the reserve material for its development; and that by mutilating the embryo in many cases a long vegetative development is obtained after which the plumule withers away, while the rootlets are very little advanced.

From the work of the above named authors the following conclusions may be drawn: (1) The endosperms of grasses are dead storehouses of reserve material at the disposal of the embryos; (2) That the endosperms are not essential for germination of the embryos.

We must bear in mind however that the authors quoted studied the germination of seeds and embryos without endosperms and cotyledons in different media and nutrient solutions, merely in their first stages of development, but that no one of them extended his study to the complete maturity of the plants developed from embryos without endosperm, together with the behavior of their offspring.

With regard to the anatomy and morphology of the maize seed, the writer would like to emphasize the fact that this question is by no means a settled one, especially that part of it which concerns the embryo. While in the majority of textbooks of botany the scutellum is described as "an haustorial suction organ of the embryo," it is not very clear where the scutellum ends and where the embryo begins; while the cotyledon of monocotyledonous plants has been reduced to a small region of a cell or cells to be seen only in the first stage of embryonic development.

Sargant and Robertson (10) claim that the scutellum of maize is distinguished by the presence of glands on its dorsal face and by the transfusion tissue connected with its vascular system. Both of these features are undoubtedly connected with its prolonged function as a suction organ.

Collins (4), after giving some of the opinions of other botanists, suggested that the plumule sheath is the coleoptyle, the region beneath the mesocotyl, and the part beneath this the radicle.

Worsdell (15) throws more light on the subject with his conclusions that the scutellum is the lamina of the cotyledon corresponding to that of the foliage leaf of the grass; the coleoptyle being part of the cotyledon, and the mesocotyl the elongated primary node.

In his experiments the present writer used kernels of *Zea indentata*, *Z. indurata*, *Z. zacharatta*, and *Z. everta*. The kernels were soaked for a few hours in warm water, and after removing the testa the scutella with the embryos were carefully detached from their endosperms.

Series of experiments were followed during the years 1917 and 1918, with embryos and scutella together, and also with embryos detached from the scutella. The results are summarized as follows:

GERMINATION IN DIFFERENT MEDIA OF EMBRYOS WITH SCUTELLA DETACHED FROM THEIR ENDOSPERMS

To test the vitality of embryos separated from endosperms, they were given a few hours, germination on water-soaked filter paper, and those that showed a beginning of germination were placed in different solutions in individual test tubes to complete the germination. As a check on this experiment, embryos were germinated also in sterilized sand, using the same solutions. The germination media were solutions, alone and in mixture, of sucrose, lactose, diastase, and agar, as well as Knop's 0.014 percent nutrient solution. The germination process was extended from 7 to 12 days at temperatures varying from 16° to 22° C.

The sucrose solutions proved to be the most satisfactory. Concentrations of 1 percent and 2 percent of sucrose produced the best germination and growth, as the following figures, which represent the average of ten plants in each case in a 7-days' germination test, show:

Embryos Germinated in	Average Length of Stem (Mesocotyl and Leaves)	Principal Root
Sucrose 1 percent	104 mm.	80 mm.
" 2 percent	100 mm.	58 mm.
" 3 percent	84 mm.	96 mm.
" 5 percent	57 mm.	80 mm.
" 10 percent	46 mm.	83 mm.
Water alone	18 mm.	34 mm.

While each of the plants produced in solutions of 1 percent and 2 percent sucrose had one principal root and two or three secondary roots poorly developed, those in 3 percent, 5 percent, and 10 percent solutions had each one principal root and many secondary roots very strong and well developed. In solutions over 5 percent, the growth was slow but vigorous. The germinations in water alone were very poor. Worthy of notice also was the very poor germination of the embryos in solution of lactose and in mixtures of sucrose, lactose, and maltose, and the slow but vigorous germination in mixtures of sucrose and diastase. In all my experiments I took the 1 percent solution of sucrose to be the best medium for the germination of the embryos.

The next step was to study the difference between the germination of complete maize kernels and the germination of embryos. This experiment also was repeated many times with different varieties of maize, using both methods of germination, in test tubes and in sterilized sand. For the germination of kernels water alone was used as a medium, while for the embryos a 1 percent solution of sucrose was employed. The best and most uniform examples were obtained with kernels and embryos of the Yellow Dent variety, soaked over night and then allowed to germinate 7 days in sterilized sand, using water for kernels and 1 percent solution of sucrose for the embryos. The results were as follows:

	Length of Stem (Mesocotyl and Leaves)	Principal Root
Kernels	100 mm.	200 mm.
Embryos	65 mm.	75 mm.

From this experiment we may observe that the embryos detached from their endosperms, when germinated, produced plants having exactly the same characteristics and features as those produced by using the whole kernels, the only difference being that they were smaller. Figure 4 better illustrates this fact.

Germination in Different Media of Embryos Detached from their Endosperms and Scutella

From kernels soaked in water from 6 to 12 hours after the testa was removed, the scutella were removed with needles from the front and sides of the embryos, and then the tissues connecting the embryo with the scutellum were cut away with a dissecting knife. The embryos thus freed had a vermiform appearance and were formed, of course, of plumule and its sheath, mesocotyl, and radicle enclosed in its sheath. For the germination of these embryos small test tubes were used, and the embryos were suspended above the solution media by means of strips of filter paper, in one set of experiments, and by small rings of paraffine in another set. These experiments were checked in sterilized sand also.

While many appropriate media were used for germination, in no case was the writer able to obtain plants with stem and radicle over 3 cm. long. It is interesting to report that in the first two days of germination a maximum growth was noticed, and that afterward the growth was very slow, many embryos withering away in from 6 to 8 days. The stem produced was the expansion of the mesocotyl and the elongation of the plumule sheath in which the plumule could be seen to be of a light green color. One principal and two secondary roots, very poorly developed, were produced. In no case was the plumule able to split its sheath and push out leaves.

It was noticed that the withering and subsequent death of some embryos was, in the majority of cases, due to decay which developed at the point where the scutellum had been detached. The decay, starting in this region, soon encircled the embryo and separated the plumule from the radicle, which then could be pulled apart.

The following table will illustrate one set of these experiments:

The kernels of *Z. everta* used in this experiment were soaked for 12 hours in water, the embryos being then removed and germinated for 48 hours. The above figures represent the averages from 4 embryos in each case.

From this table it will be noticed that the best germinations were obtained in 1.5 percent solution of sucrose, in 1 percent sucrose solution with a trace of nutrient solution, and in distilled water also with a trace of nutrient solution. Secondary roots were produced only in the 3 percent and 5 percent sucrose solutions and in the nutrient solutions. An interesting fact was the lack of germination in mixtures of sucrose, lactose, and diastase, in which the embryos began to decay before any signs of germination appeared.

FURTHER GROWTH AND DEVELOPMENT OF EMBRYOS WITH SCUTELLA (ENDOSPERMS REMOVED) AS COMPARED WITH PLANTS GROWN FROM WHOLE KERNELS

Embryos and kernels of maize, after a germination of seven days in sterilized sand, the former being watered with a 1 percent sucrose solution, and the later with water alone, were transplanted to the garden for further development. The results of similar experiments in 1917 not having been uniform on account of transplantation, the experiments were repeated in

Germination Media	Concentration		Length of		Observations
	Simple	Mixture	Stem	Radicle	
			mm.	mm.	
Sucrose	0.5%		6	7	
Sucrose	1%		2	2	
Sucrose	1.5%		12	13	
Sucrose	2%		4	5	
Sucrose	3%		3	1	Vigorous stem.
Sucrose	5%		4	1	Vigorous stem.
Sucrose	1%				
Diastase	0.02%	1.02%	3	10	
Sucrose	1%				
Lactose	0.25%				
Diastase	0.25%	1.50%	0	0	No germination
Sucrose	1%				
Agar	0.25%	1.25%	3	4	
Sucrose	1%				
Gelatin	0.25%	1.25%	4	1	
Sucrose	1%				
Nutrient solution	Trace		10	13	
Sucrose	1%				
Nutrient solution	0.007%	1.007%	5	5	
Water					
Nutrient solution	Trace		12	11	
Nutrient solution	0.007%		2	2	
Nutrient solution	0.014%		9	5	Also developed secondary roots.

1918. The embryos with scutella were planted directly in the soil in a plot prepared for this purpose. One row of 16 embryos was planted on May 15, 1918, for each of the following strains of maize: Yellow Dent, White Dent, and White Flint; and rows of 16 kernels each were also planted for each of the same strains. All kernels germinated and the first leaves appeared at the surface of the soil in seven days, the embryos requiring eight days for the appearance of leaves. The difference became more marked during the following week, at the end of which time the plants produced from the kernels were almost twice the size of those produced from the embryos.

On June 2, two plants from each row were carefully removed from the soil and observations and measurements were made. The results in average are given in the following table:

These figures also confirm the statement made above, that plants developed from embryos show the same characteristics as those developed from kernels, although being inferior in size.

After June 3, however, the individual vigor and vitality of the plants grown from the embryos began to be asserted, and by the beginning of

	Plants Developed from					
	Yellow Dent		White Dent		White Flint	
	Kernel	Embryo	Kernel	Embryo	Kernel	Embryo
Leaves (number)	5	4	5	4	5	4
Length of mesocotyl and leaves	25 cm.	13 cm.	25 cm.	13 cm.	30 cm.	17 cm.
Length of mesocotyl	4 cm.	0.5 cm.	3 cm.	1 cm.	3 cm.	1 cm.
Length of principal root	25 cm.	8 cm.	20 cm.	12 cm.	30 cm.	15 cm.
Secondary roots (number)	4	3	4	4	4	3

July a few of the plants had attained a height as great as that of the plants developed from kernels.

The first tassels appeared in July 23 on one stalk in each row of both embryos and kernels in the flint strain. Generally the appearance of tassels and silk followed the individual vigor and development of stalks rather than the ordinary behavior of each of the strains. The tasseling period was extended from July 23 to August 10. The appearance of the silk and also the pollination, as well as the whole vegetative development, were hindered by the storm of August 8 and by the cold weather which followed for a few days afterward. On account of adverse weather conditions, the silk, like the tassels, appeared irregularly, and especially it was noticed that on a few stalks which were beaten down by the storm the silk appeared late, after the pollen had been entirely shed. All the plants were in the proterandrous condition.

During the time of pollination careful observations were made in regard to the vitality of the pollen. The writer was not able to detect any difference in size, shape, and especially in the vitality of the pollen produced by the plants developed from the embryos and by those produced from kernels. The vitality of the pollen was tested by artificial pollination and by germination of the pollen in a solution of 10 per cent sucrose and 0.75 percent agar (1).

Each stalk produced at least one ear, and there was only one barren stalk which appeared in the row of the Yellow Dent variety developed from the embryo.

On account of the cold weather the maize was harvested on September 15 without being perfectly matured, and the ears were incompletely developed. Some of the observations in regard to the number of internodes, length and diameter of stalks, etc., are given in the table following, the figures representing the average in each case taken from six typical stalks in each row:

From this table we observe that in each case the stalks developed from embryos were shorter than those developed from kernels, and this fact is explained by the decrease in the number of internodes which appeared in the stalks developed from embryos. Also the diameter of stalks was smaller in the plants developed from embryos. While there seemed to be no rule governing the node on which the ear appeared, it was noticed,

	Plants Developed from					
	Yellow Dent		White Dent		White Flint	
	Kernel	Embryo	Kernel	Embryo	Kernel	Embryo
Length of stalks (meters)	1.65	1.46	1.81	1.72	1.26	1.18
Internodes (number)	18	16	22	19	12	12
Diameter of stalks (cm.)	1.93	1.35	2	1.80	1.10	1.00
Ear developed at node	10	9	16	13	7	5

however, that in the stalks produced from embryos the ears appeared at a lower node than in other stalks, and that, while no ear was perfectly developed, those on the plants produced from the embryos were the best, the explanation being that pollination and fecundation occurred after the storm and cold weather of early August while in the case of the plants developed from kernels, pollination and fecundation occurred previous to and during the storm.

Discussion

This problem, as stated at the beginning of the article, is one which will require a study extending through several generations of maize in order to draw definite conclusions. The writer believes, however, that the results obtained from these preliminary experiments offer material for at least a discussion.

Sufficient proof is at hand to demonstrate that with maize, normal plants may be developed from embryos without endosperms. The germination of embryos with their endosperms removed seems to follow through exactly the same stages as those of the germination of whole seeds, except that the process is slower. The lack of the endosperm in germination does not so much influence the root system of the young plants as it produces a general retarding influence on the whole development.

The tendency toward a longer period of growth of plants developed from embryos with endosperms removed is reduced by the tendency of these plants to reduce their number of internodes.

The chemical composition of different anatomical parts of the maize kernel also must be considered. From the well-known work of Hopkins (7) the writer has compiled the following table:

	Percentage of Total in Kernel			
	Protein	Oil	Carbohydrates	Ash
Embryo	20.14	82.43	4.97	74.55
Endosperm	76.66	15.18	86.68	21.33
Testa	2.07	1.08	6.80	3.06
Tip Cap	1.14	0.69	1.56	1.06

From this table it will be seen that the embryo contains almost all the oil of the kernel and the largest percentage of minerals, while the endosperm contains almost all the carbohydrates and protein of the kernel. Theoretically, therefore, the slower germination of the embryos with endosperms removed is explained by the lack of carbohydrates. Practically, however, while vigorous plants can be produced from embryos the endosperms of which have been removed, the fact is demonstrated that the small percentage of protein and of carbohydrates which the embryo contains is sufficient to insure the germination and further development of the plants.

We can not deny, however, that the presence of endosperms is beneficial in the process of germination, as well as in the further development of the plants.

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EXPLANATION OF PLATE XLI

FIG. 1. *Zea indentata,* Yellow Dent variety. Kernels, median longitudinal section. × 2½.

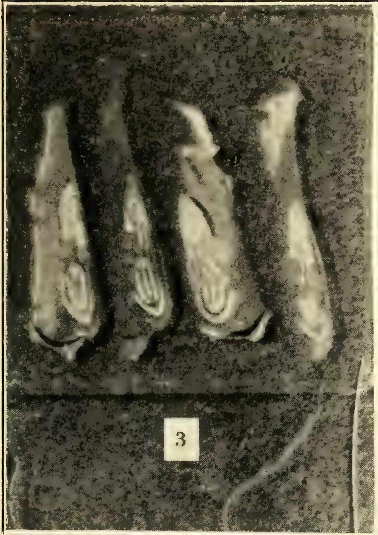
FIG. 2. *Z. indentata,* White Dent variety. Kernel after 10 hours' germination, fixed in alcohol; free-hand median longitudinal section, cleared in glycerine, mounted in glycerine-jelly. × 2½.

FIG. 3. *Z. indentata*, White Dent variety. Embryos after 8 hours' germination; free-hand median longitudinal section, cleared in chloral hydrate. Note the scutella and the embryos with plumule, mesocotyl, and hypocotyl or radicle. Note also the origin of dermatogen, periblem, and plerome in roots, and the first endogenous adventitious roots. × 2½.

FIG. 4. *Z. indentata*, Yellow Dent variety. Plants developed from whole kernel (left), and from embryo with the endosperm removed (right), after seven days' germination. 4/10 natural size.

FIG. 5. *Z. indentata*, White Dent variety. Mature plants developed from kernel (left), and from embryo with the endosperm removed (right).

FIG. 6. *Z. indentata*, White Dent variety. Ears produced by plant developed from whole kernel (left), and by plants developed from an embryo with the endosperm removed (right). ½ natural size.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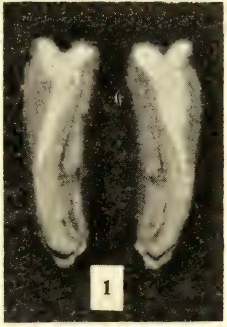
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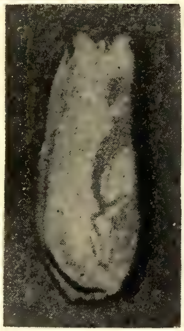
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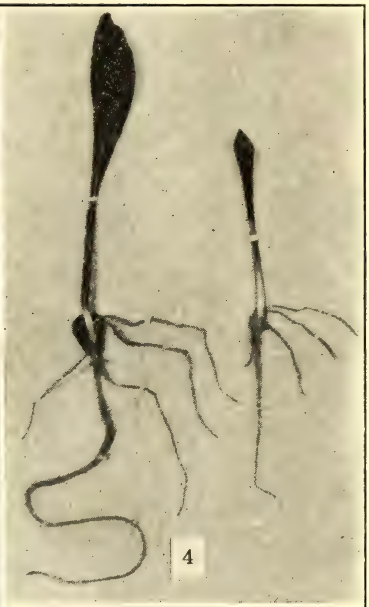
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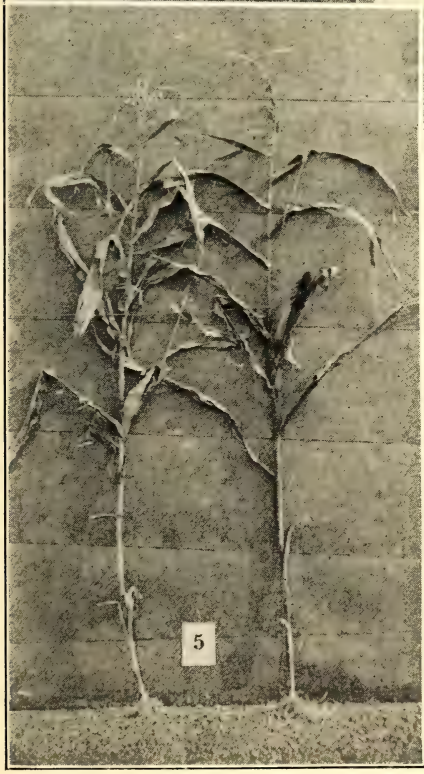
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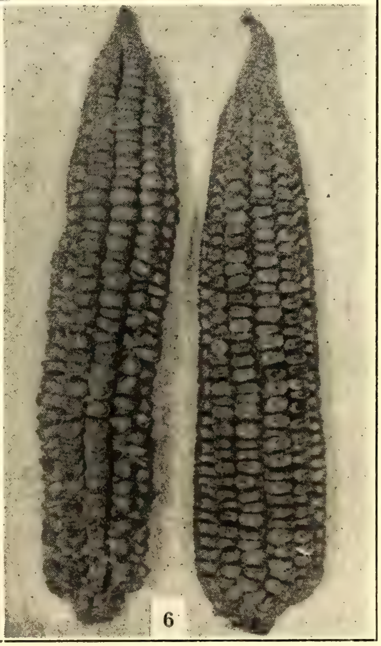
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ANDRONESCU: GERMINATION OF ZEA MAY.

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